Effects of Nicardipine on Ischemic Mechanical Failure and Tissue Injury in Isolated Perfused Rat Heart

Hisataka SHIKAMA, Ph.D., Osamu NOSHIRO, B.Sc., Akiko OHTA, B.Sc., and Isao OHHATA, Ph.D.

SUMMARY

Using a Langendorff rat heart preparation, we examined effects of nicardipine, a calcium channel blocker, on different stages of ischemic damage, characterized by a development of contracture and leakage of intracellular enzymes. Maximum recoveries of heart rate (HR) and peak left ventricular pressure-HR product after 20 min ischemia were attenuated by about 25% compared with those before ischemia. When nicardipine (0.1 μmol) was added to the perfusate 5 min prior to ischemia, this mechanical failure recovered completely to the pre-ischemic level. Although a significant increase in left ventricular end-diastolic pressure was observed in hearts exposed to 30 min ischemia, the amount of creatine kinase (CK) released during re-flow after 30 min ischemia was not enhanced by contracture but was proportional to the duration of ischemia (compared with that of 20 min ischemia). Nicardipine reduced CK leakage by 25% after 30 min ischemia but did not alter either ATP levels or coronary flow. The beneficial effects of nicardipine on ischemic damage are probably related to inhibition of calcium influx (Terai et al: Biochem Pharmacol 30: 375, 1981), which may accompany reperfusion of ischemic myocardium.

Additional Indexing Words:
Isolated rat heart Ischemia Nicardipine

MYOCARDIAL ischemia causes irreversible impairment of mechanical function and metabolic changes in the heart, which increase with the duration of ischemia. One aspect which shows post-ischemic damage is a development of contracture (i.e., elevated left ventricular end-diastolic

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Loss of intracellular enzymes on reperfusion after ischemia is also a striking feature of irreversible damage. However, it is not known precisely how these two indices contribute to mechanical failure of the heart exposed to differing periods of ischemia.

The mechanisms responsible for ischemic damage are not clear, but there are several hypotheses that relate increased cellular accumulation of calcium and/or depletion of ATP. Calcium channel blockers (e.g., verapamil, diltiazem) are thought to block calcium influx and to prevent the accumulation of calcium in mitochondria during ischemia and reperfusion. On the other hand, nifedipine showed ATP-sparing effects after ischemia, but this effect has not been replicated. These findings, along with clinical studies, suggest that a calcium channel blocker is beneficial in treatment for ischemic diseases of the heart.

Nicardipine, a 1,4-dihydropyridine derivative, is a potent cerebral and coronary vasodilator with hypotensive activity. Nicardipine inhibits radioactive calcium uptake in the rabbit aorta. In this study, we examined effects of nicardipine on different stages of ischemic damage, which were characterized by a development of contracture and leakage of intracellular enzymes in isolated rat heart. The results showed that nicardipine improved mechanical function and reduced enzyme leakage from hearts exposed to ischemia.

**Materials and Methods**

*Animals*

Male Sprague-Dawley rats weighing 350–400 Gm were used. The animals were deprived of food overnight but were allowed free access to water. Anesthesia was induced with pentobarbital (4.5 mg/100 Gm body wt) injected intraperitoneally.

*Perfusion apparatus*

The perfused heart was prepared by a modified Langendorff method, which included cannulation of the aorta and connection to a constant work load of 60 mmHg. The perfusion medium was a Krebs-Henseleit bicarbonate buffer containing 11 mM glucose, equilibrated with a gas mixture of 95% O₂ + 5% CO₂. The perfusate was not recycled in a flow-through system. The temperature of the preparation was maintained at 37°C with double-layered water jackets.

*Time sequence of experiments*

Initially, rat hearts were perfused for 15 min (the control period) with oxygenated Krebs-Henseleit bicarbonate buffer. Then they were submitted to global ischemia (zero mmHg) for either 20 min or 30 min. After ischemia,
aerobic perfusion was restored for 30 min. Samples of the coronary effluent were collected at selected intervals and analyzed for creatine kinase (CK) and lactate. Generally, nicardipine (0.1 μmol) was infused for the last 5 min of the control period. In some experiments, additional infusion of nicardipine was done during the reperfusion period (Tables II and III). In order to measure intracellular concentrations of ATP, ADP, and AMP at the end of reperfusion, a heart was freezed-clamped at the temperature of liquid nitrogen and pulverized by a mortar cooled with liquid nitrogen.

**Mechanical function**

Intraventricular pressure was measured by inserting a needle through the apex of the heart into the left ventricle. Heart rate was measured with a frequency counter fed on the output of the left ventricular pressure. Left ventricular pressure (systolic-diastolic) was used as an index of contractile function.

**Analytical methods**

Adenine nucleotides, ATP\(^{12}\) and ADP, AMP\(^{13}\) were determined enzymatically. Concentration was expressed as μmol/Gm dry wt. Adenylate energy charge was calculated following the equation proposed by Atkinson.\(^{14}\)

\[ EC = \frac{(ATP+0.5 ADP)}{(ATP+ADP+AMP)} \]

Creatine kinase was measured by the method of Urdal and Stromme.\(^{15}\) Lactate was measured enzymatically.\(^{16}\)

**Chemicals**

Nicardipine hydrochloride was obtained from Yamanouchi Pharmaceutical Co., Ltd., Tokyo. Reagents for the determination of adenine nucleotides, CK, and lactate were from Boehringer-Mannheim-Yamanouchi Co., Ltd., Tokyo. Other reagents were of analytical grade from commercial sources.

**RESULTS**

**Mechanical performance**

Changes in peak left ventricular pressure and left ventricular end-diastolic pressure of hearts exposed to differing periods of ischemia

Changes in peak left ventricular pressure (LVP) and left ventricular end-diastolic pressure (LVEDP) were observed during a 30 min reperfusion period after 20 min (Fig. 1-A) and 30 min (Fig. 1-B) ischemia. LVP during reperfusion was similar in both groups. Hearts exposed to 20 min ischemia showed no significant increase in LVEDP. But, a 30 min ischemic period elicited an increase in LVEDP (Fig. 1-B). LVEDP increased significantly up to 43.3±6.1 mmHg at 5 min (p<0.01). This rise continued until 20 min of
Fig. 1. Changes in peak left ventricular pressure (LVP) and left ventricular end-diastolic pressure (LVEDP) of hearts exposed to differing periods of ischemia. Hearts were perfused for 15 min (the control period), followed by 20 min (A) or 30 min (B) of total global ischemia. Subsequently, hearts were reperfused for 30 min under non-ischemic conditions. Each point represents mean ± SE. Numbers of observations are shown in parentheses. *p < 0.05, compared with the value before ischemia.

Effect of nicardipine on recoveries of heart rate and pressure-rate product during reperfusion after 20 min ischemia

Although 20 min ischemia showed little effect on contractile function estimated by left ventricular pressure (LVP-LVEDP) (Fig. 1), maximum recoveries of heart rate (HR) and product of LVP and HR (pressure-rate product) after 20 min ischemia were attenuated by about 25% (24% and 29%, respectively) compared with their values before ischemia (Fig. 2). On the other hand, HR and the pressure-rate product of nicardipine-treated hearts recovered their pre-ischemic values.

Enzyme leakage

Changes in creatine kinase leakage and coronary flow of hearts exposed to differing periods of ischemia

The results obtained from Figs. 1 and 2 suggested that irreversible mechanical impairment had began earlier than an increase in LVEDP. To obtain additional evidence that irreversible ischemic damage had already occurred within 20 min, we measured creatine kinase (CK) leakage, which has long been regarded as a sensitive index of ischemic tissue damage. As
Fig. 2. Effects of nicardipine on recoveries of heart rate (HR) and pressure-rate product (LVP×HR) during reperfusion after 20 min ischemia. After a 10 min control period, hearts were perfused with (●) or without (○) nicardipine at 0.1 μmol for another 5 min. At the end of 20 min ischemia, the perfusate was switched to the normal buffer and perfusion pressure was raised to 60 mmHg, so that hearts could be reperfused. Values are shown mean±SE. *p<0.05, compared with control at the same interval. +p<0.05, compared with the value before ischemia.

Table I. Changes in Creatine Kinase Leakage and Coronary Flow of Hearts Exposed to Differing Periods of Ischemia

<table>
<thead>
<tr>
<th>Period of ischemia</th>
<th>Creatine kinase (CK) (U)</th>
<th>Rate of CK leakage (U/min of ischemia)</th>
<th>Coronary flow (CF) (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 min (5)</td>
<td>13.51±0.75</td>
<td>0.676±0.038</td>
<td>169.3±7.5</td>
</tr>
<tr>
<td>30 min (7)</td>
<td>20.45±1.14*</td>
<td>0.682±0.038</td>
<td>109.5±7.0*</td>
</tr>
</tbody>
</table>

Samples of the coronary effluent were collected at selected intervals during 20 min reperfusion and analyzed for CK activity. * p<0.05, compared with 20 min ischemia. Numbers of observations are shown in parentheses.

shown in Table I, hearts exposed to 30 min ischemia released more CK than did hearts exposed to 20 min ischemia (p<0.05). There was also a marked reduction of coronary flow (CF) in hearts treated with 30 min ischemia (p<0.05). However, the mean rate of CK leakage over its respective period of ischemia was very similar in both groups (0.676±0.038 U/min for 20 min ischemia vs. 0.682±0.038 U/min for 30 min ischemia, Table I). Thus, ischemic tissue injury estimated by CK leakage was not enhanced by a rise in LVEDP, but was proportional to the duration of ischemia.

Effects of nicardipine on changes in CK, lactate and CF induced by 30 min ischemia
Table II. Effects of Nicardipine on Changes in Creatine Kinase, Lactate and Coronary Flow Induced by 30 Min Ischemia

<table>
<thead>
<tr>
<th>Condition</th>
<th>Creatine kinase (U)</th>
<th>Lactate (μmol)</th>
<th>Coronary flow (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemia (7)</td>
<td>20.45±1.14</td>
<td>47.3±3.3</td>
<td>109.5±7.0</td>
</tr>
<tr>
<td>Ischemia + nicardipine (5)</td>
<td>15.11±0.99*</td>
<td>44.7±2.0</td>
<td>110.7±14.4</td>
</tr>
</tbody>
</table>

After 10 min of the control period, hearts were perfused with or without nicardipine at 0.1 μmol for another 5 min. Additional infusion of nicardipine was done during 20 min reperfusion period. Nicardipine increased coronary flow by 30% at the end of 5 min drug infusion before ischemia. A 30 min ischemia alone decreased coronary flow from the pre-ischemic control value of 13.4±0.7 ml/min to 8.3±0.7 ml/min at the end of 20 min reperfusion. Numbers of observations are shown in parentheses. *p<0.05.

Table III. Effects of Nicardipine on Changes in Myocardial Concentrations of Adenine Nucleotides Induced by 30 Min Ischemia

<table>
<thead>
<tr>
<th>Condition</th>
<th>ATP (μmol/Gm dry wt)</th>
<th>ADP (μmol/Gm dry wt)</th>
<th>AMP (μmol/Gm dry wt)</th>
<th>EC</th>
<th>Total adenine nucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (4)</td>
<td>22.2±1.3</td>
<td>4.2±0.3</td>
<td>0.9±0.1</td>
<td>0.891±0.013</td>
<td>27.3±1.0</td>
</tr>
<tr>
<td>Ischemia (7)</td>
<td>10.7±0.6</td>
<td>2.6±0.5*</td>
<td>1.5±0.1*</td>
<td>0.812±0.018*</td>
<td>14.7±0.6*</td>
</tr>
<tr>
<td>Ischemia + nicardipine (5)</td>
<td>9.9±1.7*</td>
<td>1.8±0.2*</td>
<td>1.4±0.2*</td>
<td>0.834±0.017*</td>
<td>13.1±0.5*</td>
</tr>
</tbody>
</table>

Control hearts were perfused with normal buffer for 45 min under non-ischemic conditions. Other experimental conditions were shown in Table II. Numbers of observations are shown in parentheses. *p<0.05, compared with control.

Nicardipine decreased CK leakage from the 30 min ischemic control value of 20.45±1.14 U/heart to 15.11±0.99 U/heart (p<0.05, Table II). Under the same conditions this agent did not significantly alter either CF or lactate release during reperfusion. Thus, it is unlikely that the nicardipine induced reduction of CK leakage was due to a vasodilating effect.9)

Effects of nicardipine on changes in myocardial concentrations of adenine nucleotides induced by 30 min ischemia

There is much evidence that ATP depletion accelerates ischemic injury.9) To investigate a possible relationship between ATP concentration and the reduction of CK leakage by nicardipine, we measured adenine nucleotide concentrations at the end of reperfusion (Table III). With 30 min ischemia and reperfusion, there were substantial reductions of myocardial ATP concentration (more than 50%), adenylate energy charge (EC, 8.3%) and total amounts of adenine nucleotides (ATP+ADP+AMP, 46.1%). However, nicardipine did not improve these three parameters.
DISCUSSION

Using a Langendorff rat heart preparation, we examined effects of nicardipine on ischemic damage induced by various lengths of total global ischemia. The results showed that nicardipine-treated hearts recovered stable mechanical function after 20 min ischemia, when hearts perfused without this agent failed to recover pre-ischemic values (Fig. 2-A and B). Similar beneficial effects on mechanical failure after ischemia have been reported by other calcium channel blockers such as nifedipine, verapamil and diltiazem.17)-20)

Current studies suggest that deleterious effects of ischemia are characterized by early falls in cellular concentrations of high energy phosphates (ATP and creatine phosphate)21) and by transient episodes of left ventricular failure.22) At much later stages, this damage is evidenced by a major leakage of intracellular enzymes (e.g., CK, lactate dehydrogenase) and by a myocardial contracture.23) If myocardium is reperfused at the time when significant cellular damage has already occurred, reperfusion itself may cause an acceleration of tissue damage characterized by a massive loss of intracellular enzymes.

In accord with these current studies, ischemia, maintained at 37°C for 30 min, resulted in a rigid state, which was evidenced by a rise in LVEDP during reperfusion (Fig. 1-B). Hearts exposed to 30 min ischemia released more CK than did hearts exposed to 20 min ischemia (Table I). However, the mean rate of CK leakage over its respective period of ischemia was similar in both groups. Thus, ischemic tissue injury estimated by CK leakage was not enhanced by a rise in LVEDP but was proportional to the duration of ischemia.

Nicardipine reduced CK leakage significantly by 26% after 30 min ischemia and reperfusion (Table II). According to reports of Ichihara and Abiko20) and Bersohn and Shine,18) calcium channel blockers such as diltiazem and verapamil improved mechanical impairment after ischemia through their ATP-sparing effects.24) However, little is known about the mechanisms responsible for reduction of enzyme leakage by calcium channel blockers.

We, firstly, examined effects of nicardipine on CF during reperfusion. In contrast to the significant increase in CF induced by nicardipine under non-ischemic conditions (see the legend of Table II), this agent had no effect on CF after ischemia (Table II). Therefore, it is unlikely that the effect of nicardipine on reduction of CK leakage was due to vasodilating activity.25,26) Secondly, nicardipine failed to alter adenine nucleotide metabolism after ischemia (Table III), since ATP concentrations, EC and the total amount
of adenine nucleotides were not changed by this agent. Furthermore, diltiazem at the same concentration also failed to alter these parameters under the same conditions (Shikama et al, unpublished observations). However, Weishaar and Bing\textsuperscript{19}) reported that diltiazem at 0.4 mM reduced CK leakage by one-half compared with 120 min ischemic hearts without the drug. They also observed that creatine phosphate was restored to near-normal levels, when ATP concentration was not increased. Clearly, further study is needed to assess effects of nicardipine on the recovery of high energy phosphate synthesis after ischemia. However, the cytosolic compartmentalization of ATP proposed by Bricknell and Opie\textsuperscript{27}) may explain why hearts perfused with nicardipine released less enzymes without changes in ATP levels on reperfusion.

Finally, another important mechanism which causes ischemic damage is an overload of intracellular calcium on reperfusion.\textsuperscript{28}) As mentioned above, nicardipine inhibits calcium influx.\textsuperscript{10}) Therefore, it is highly possible that this agent maintained the integrity of the plasma membrane and reduced CK leakage through this mechanism. These data are consistent with the proposed beneficial effects of calcium channel blockers on ischemic damage\textsuperscript{17)—20}) and indicate that nicardipine could be useful in the treatment of myocardial ischemic diseases.

**References**

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