Protective Effects of Ranolazine, a Novel Anti-ischemic Drug, on the Hydrogen Peroxide-Induced Derangements in Isolated, Perfused Rat Heart: Comparison With Dichloroacetate

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ABSTRACT—The effect of ranolazine, a novel anti-ischemic drug that stimulates the activity of pyruvate dehydrogenase, on hydrogen peroxide (H₂O₂)-induced mechanical and metabolic derangements was studied in isolated rat heart and compared with that of dichloroacetate (DCA), an activator of pyruvate dehydrogenase. The heart was perfused aerobically by the Langendorff’s technique at a constant flow and driven electrically. H₂O₂ (600 μM) decreased the left ventricular developed pressure and increased the left ventricular end-diastolic pressure (i.e., mechanical dysfunction), decreased the tissue level of adenosine triphosphate (i.e., metabolic derangement), and increased the tissue level of malondialdehyde (MDA) (i.e., lipid peroxidation). These mechanical and metabolic derangements induced by H₂O₂ were significantly attenuated by ranolazine (10 or 20 μM). On the other hand, DCA (1 mM) was ineffective in attenuating the H₂O₂-induced mechanical and metabolic derangements. Ranolazine, however, did not modify the tissue MDA level, which was increased by H₂O₂. In the normal (H₂O₂-untreated) heart, ranolazine did not alter the mechanical function and energy metabolism. These results demonstrate that ranolazine attenuates mechanical and metabolic derangements induced by H₂O₂. It is suggested that the protective action of ranolazine against the H₂O₂-induced derangements is due to neither the energy-sparing, DCA-like, nor anti-oxidant effects.

Keywords: Ranolazine, Dichloroacetate, Hydrogen peroxide, Lipid peroxidation, Rat heart

It has been generally accepted that the primary mechanism of action of anti-anginal drugs is improvement of the myocardial oxygen balance between supply and demand by either an increase in coronary flow or a decrease in cardiac mechanical function, or both. Therefore, nitrates, β-adrenoceptor antagonists and Ca²⁺ channel blockers have been used widely for treatment of patients with ischemic heart disease. Recently, ranolazine [(±)-N-(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy)propyl]-1-piperazine acetamide] (Fig. 1) has been demonstrated to attenuate the ischemic derangements in vivo (1, 2) and in vitro (3–5) in animal models. Similar cardioprotective action of ranolazine has been demonstrated in patients with angina pectoris (6, 7). Interestingly, ranolazine is effective in attenuating ischemic derangements even when it does not exert a significant action on hemodynamics, and therefore the anti-ischemic action of ranolazine is probably not due to improvement of the myocardial oxygen balance (4, 5). Recently, McCormack et al. (5) have demonstrated that the protective action of ranolazine may be, at least in part, due to stimulation of glucose oxidation and reduction of fatty acid oxidation through activation of pyruvate dehydrogenase, which regulates the entry of pyruvate into the tricarboxylic acid cycle. Nevertheless, the mechanism of the anti-ischemic effect of ranolazine is not fully understood.

Reactive oxygen species, such as superoxide anion, hydroxyl radical and hydrogen peroxide (H₂O₂), have been shown to participate in ischemia-reperfusion damage (8–10). These reactive oxygen species are generated intra- and extracellularly in the myocardium and endothelium during ischemia and reperfusion, causing lipid peroxidation of the cell membrane, mechanical dysfunction and metabolic derangement of the heart (8–10). Therefore, many studies have addressed the action of
substances that protect the myocardium from reactive oxygen-induced damage. For example, the radical scavengers or anti-oxidants have been shown to protect the myocardium against oxidative stress (11–13). We have found that some of the anti-ischemic drugs, such as lidocaine (14), dilazep (15) and prazosin (16), attenuate the mechanical dysfunction and metabolic changes induced by \( \text{H}_2\text{O}_2 \) in isolated perfused rat heart. Nevertheless, it is unclear whether ranolazine attenuates the myocardial changes induced by reactive oxygen species. The present study, therefore, was carried out to investigate the effects of ranolazine on the mechanical and metabolic derangements induced by exogenous \( \text{H}_2\text{O}_2 \) in the isolated perfused rat heart and compared them with those of dichloroacetate (DCA), an activator of pyruvate dehydrogenase (17–19).

**MATERIALS AND METHODS**

**Heart perfusion**

The protocol of animal experiments in the present study was approved by the “Asahikawa Medical College Committee on Animal Research”. Male Sprague-Dawley rats (9- to 10-week-old; Sankyo Labo Service Corporation, Sapporo) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) 20 min after an injection with heparin (1,000 units/kg, i.p.). After thoracotomy, the hearts were rapidly removed, and then retrograde perfusion was started using a cannula inserted into the aorta according to the Langendorff’s technique. The perfusion buffer was a Krebs-Henseleit bicarbonate (KHB) buffer containing 118 mM NaCl, 4.7 mM KCl, 1.2 mM KH\(_2\)PO\(_4\), 1.2 mM MgSO\(_4\), 2.5 mM CaCl\(_2\), 25 mM NaHCO\(_3\) and 11 mM glucose equilibrated with a gas mixture of 95% O\(_2\) + 5% CO\(_2\) and maintained at 37°C. The oxygen tension of the buffer measured by a blood gas analyzer (Model 813; Instrumentation Laboratory, Lexington, MA, USA) was about 550 mmHg. The hearts were initially perfused at a constant perfusion pressure of 80 cmH\(_2\)O. About 10 min after constant pressure perfusion, perfusion was switched to the constant flow perfusion (10 ml/min) using a microtube pump (Eyela MP-A; Tokyo-Rikakikai Instruments, Tokyo), and this flow rate was maintained throughout the experiment. The heart rate of spontaneously beating hearts was about 270 beats/min and was kept constant by pacing the heart at 300 beats/min with an electronic stimulator (3F46; San-Ei Instruments, Tokyo) during the course of the study. Rectangular pulses having 2-msec duration with the voltage of 6 V (about 3 times the threshold voltage) were applied to the left ventricle for pacing of the heart.

As indices of mechanical function, left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP) and left ventricular developed pressure (LVDP) were employed. The values of LVSP and LVEDP were determined from the left ventricular pressure (LVP) curves recorded continuously during the course of the study, and the LVDP value was calculated as LVSP minus LVEDP. For measurement of LVP, a saline-filled polyethylene cannula, connected to a pressure transducer, was inserted into the left ventricular cavity via the left atrium. Another pressure transducer was connected to the aortic cannula for recording of coronary perfusion pressure (CPP). Before the start of the experiment, the heart was allowed to stabilize for 20 min under the conditions of constant flow perfusion.

**Experimental protocol**

The heart was perfused with KHB buffer at a constant flow throughout the experiment (stabilization period for 20 min and observation period for 38 min). During the observation period, \( \text{H}_2\text{O}_2 \) and/or a drug (ranolazine or DCA) were infused into the aortic cannula in which KHB buffer flowed. The effects of ranolazine or DCA on the mechanical function and energy metabolism in both \( \text{H}_2\text{O}_2\)-treated heart and \( \text{H}_2\text{O}_2\)-untreated heart (normal heart) experiments were examined. In the \( \text{H}_2\text{O}_2\)-treated heart experiments, the hearts were divided into five groups: vehicle, ranolazine (5 \( \mu \)M), ranolazine (10 \( \mu \)M), ranolazine (20 \( \mu \)M) and DCA groups. In these groups, ranolazine, DCA or vehicle (KHB buffer) was infused into the aortic cannula for 18 min at a constant flow rate of 0.1 ml/min. \( \text{H}_2\text{O}_2 \) was infused into the aortic cannula at the constant flow rate of 0.1 ml/min for 3 min from 5 min after the start of infusion of ranolazine, DCA or vehicle. In the normal heart experiments, the hearts were divided into five groups: vehicle, ranolazine (5 \( \mu \)M), ranolazine (10 \( \mu \)M), ranolazine (20 \( \mu \)M) and DCA groups. The ex-
perimental conditions and protocol in the normal heart experiments were essentially the same as those in the \( \text{H}_2\text{O}_2 \)-treated heart experiments, except for an infusion of saline solution instead of \( \text{H}_2\text{O}_2 \) solution. In each group, LVSP, LVEDP and CPP were continuously recorded over a 38-min observation period. To measure the tissue levels of high-energy phosphates, the heart was frozen at the end of the observation period (38 min after the start of infusion of ranolazine, DCA or vehicle) with freezing clamps previously chilled in liquid nitrogen.

Some hearts in the vehicle group in the normal heart experiments and those in the vehicle and ranolazine (20 \( \mu \text{M} \)) groups in the \( \text{H}_2\text{O}_2 \) heart experiments were frozen 10 min after the end of \( \text{H}_2\text{O}_2 \) infusion (18 min after the start of infusion of ranolazine or vehicle) for measurement of the tissue level of malondialdehyde (MDA).

**Biochemical analyses**

The frozen myocardial samples were stored in liquid nitrogen (at \(-196^\circ\text{C}\)) until the biochemical analysis was performed. The frozen myocardial sample was pulverized in a mortar cooled with liquid nitrogen. A part of the pulverized tissue powder was weighed and put into an oven and allowed to stand in it overnight, to measure the tissue water content and dry weight of the tissue. The remainder of the tissue powder was used for determination of the tissue levels of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), creatine phosphate (CrP) and MDA.

ATP, ADP, AMP and CrP were extracted from the pulverized tissue sample with 6% perchloric acid. The tissue extract was centrifuged at 10,000 \( \times g \) for 10 min at 4\(^\circ\text{C}\). The supernatant was neutralized by 70% KOH and centrifuged at 10,000 \( \times g \) for 10 min at 4\(^\circ\text{C}\) again. The resultant solution was used for determination of ATP, ADP, AMP and CrP. These metabolites were measured according to the standard enzymatic procedures (20).

MDA, a product of lipid peroxidation, was measured by high-performance liquid chromatography (HPLC) according to the method described by Koller and Bergmann (12). MDA was extracted from the pulverized tissue sample with 10 mM phosphate buffer (pH 8.0), and the tissue extract was centrifuged at 10,000 \( \times g \) for 10 min at 4\(^\circ\text{C}\), and the supernatant was filtered through a millipore filter (UFC31CC; Nihon Millipore Kogyo K.K., Yonezawa) at 2,000 \( \times g \). The filtered solution (20 \( \mu l \)) was injected into the HPLC system (LC-9A; Shimadzu Corporation, Kyoto), equipped with an ODS guard column (0.4 \( \times \) 1 cm, Shimadzu Corporation) and an ODS column (0.46 \( \times \) 25 cm, Shimadzu Corporation). MDA was separated with 15% acetonitrile containing 50 mM myristyltrimethylammonium bromide and 1.0 mM NaHPO\(_4\) adjusted to pH 6.8, at a flow rate of 1.0 ml/min. The effluent was moni-
tored at 267 nm using a spectrophotometric detector (SPD-2AS, Shimadzu Corporation). The quantitative analysis was performed by comparison with standard curves. MDA standards were prepared by acid hydrolysis of malonaldehyde bis(dimethyl acetal). Before measurement of the tissue MDA level, we confirmed that ranolazine did not interfere with measurement of MDA. The mixture consisting of various concentrations of MDA standards and ranolazine (20 \( \mu \text{M} \)) in the 10 mM phosphate buffer (pH 8.0) was incubated for 20 min. The concentration of MDA in the buffer in the presence or absence of ranolazine was measured by HPLC according to the method described above.

**Drugs**

Ranolazine dihydrochloride (Kissei Pharmaceutical Co., Ltd., Matsumoto) and sodium DCA (Tokyo Kasei Co., Ltd., Tokyo) were dissolved in KHb buffer solution. \( \text{H}_2\text{O}_2 \) (Nacalai Tesque, Ltd., Kyoto) was diluted with saline solution. Ranolazine, DCA or vehicle (KHb buffer) solution was infused at a flow rate of 0.1 ml/min using an infusion pump into the inflow tube connected to the side arm of the aortic cannula. \( \text{H}_2\text{O}_2 \) or saline solution was also infused to the inflow tube at a flow rate of 0.1 ml/min using another infusion pump. The final concentration of ranolazine in the perfusate was set to 5, 10 or 20 \( \mu \text{M} \), that of DCA in the perfusate was set to 1 mM and that of \( \text{H}_2\text{O}_2 \) in the perfusate was set to 600 \( \mu \text{M} \). The reagents and enzymes used for biochemical analysis were purchased from Sigma Chemical Company (St. Louis, MO, USA) or Aldrich Chemical Company (Milwaukee, WI, USA).

**Statistical analyses**

All values are expressed as the mean \( \pm \) S.E.M. When changes of LVSP, LVEDP, LVDP and CPP were compared between vehicle-treated and drug-treated groups, statistical analysis was performed with a two-way repeated measures ANOVA followed by Dunnett's test for multiple comparisons. If a significant difference was obtained between these groups, further comparisons at each time point were performed by Dunnett's test (Figs. 2, 3 and 4). When data of the tissue levels of energy metabolites were compared between vehicle-treated and drug-treated groups, statistical analysis was performed with a two-way factorial ANOVA followed by Dunnett's test for multiple comparisons (Fig. 5). Data of the tissue level of MDA were analyzed with a one-way ANOVA followed by Dunnett's test for multiple comparison (Table 1). A difference was considered statistically significant at \( P < 0.05 \).
RESULTS

Effect of ranolazine on mechanical function and coronary resistance

In the normal (H$_2$O$_2$-untreated) heart, there was no significant difference in the values of LVSP (P=0.334 by ANOVA), LVEDP (P=0.399 by ANOVA) and CPP (P=0.208 by ANOVA) among the vehicle, ranolazine (5 μM), ranolazine (10 μM), ranolazine (20 μM) and DCA groups (data not shown), suggesting that neither ranolazine nor DCA modify the mechanical function and coronary resistance in the normal heart. Figure 2 shows the effects of ranolazine and DCA on the H$_2$O$_2$-induced changes in LVSP and LVEDP. Before starting the H$_2$O$_2$ infusion, there was no significant difference in the values of LVSP and LVEDP between the vehicle and ranolazine groups and between the vehicle and DCA groups. In the vehicle group, H$_2$O$_2$ decreased LVSP temporarily but markedly (P<0.001 by ANOVA). The LVSP, which had been decreased by H$_2$O$_2$, increased after the end of H$_2$O$_2$ infusion and returned to the initial level. The H$_2$O$_2$-induced changes in LVSP were not significantly different among the five groups (P=0.644 by ANOVA). H$_2$O$_2$ also increased LVEDP markedly (P<0.001 by ANOVA), and the increase in LVEDP was accompanied by an increase in LVSP. The increase in LVEDP induced by H$_2$O$_2$ was prevented almost completely by ranolazine (10 and 20 μM) (P<0.05 by ANOVA followed by Dunnett's test); this beneficial effect of ranolazine (10 and 20 μM) was observed at 13 or 18 to 38 min after starting the ranolazine infusion (P<0.05). However, a low concentration of ranolazine (5 μM) and DCA (1 mM) failed to attenuate the H$_2$O$_2$-induced increase in LVEDP.

Figure 3 shows the effect of ranolazine on the H$_2$O$_2$-

![Diagram showing changes in LVSP and LVEDP](image)

**Fig. 2.** Effects of ranolazine (5, 10 and 20 μM) and DCA (1 mM) on the H$_2$O$_2$-induced changes in mechanical function. The changes of LVSP (upper panel) and LVEDP (bottom panel) in the vehicle (○, n=6), ranolazine (5 μM) (●, n=7), ranolazine (10 μM) (△, n=6), ranolazine (20 μM) (▲, n=6) and DCA (■, n=5) groups are shown. Each value represents a mean±S.E.M. *P<0.05, when compared with the value in the vehicle group.
induced changes in LVDP. Before starting the H$_2$O$_2$ infusion, there was no significant difference in the LVDP value between the vehicle and ranolazine groups. In the vehicle group, H$_2$O$_2$ decreased LVDP markedly (P<0.001 by ANOVA). Ranolazine (10 and 20 μM) significantly attenuated the H$_2$O$_2$-induced decrease in LVDP (P<0.05 by ANOVA followed by Dunnett’s test), although it did not attenuate it at the low concentration (5 μM); this beneficial effect of ranolazine (10 and 20 μM) was observed at 18 to 38 min after starting the ranolazine infusion (P<0.05). At the end of experiment ("38 min" in Fig. 3), the values of LVDP in the ranolazine (5 μM), ranolazine (10 μM) and ranolazine (20 μM) groups were about 1.3, 2.4 and 2.5 times the value in the vehicle group.

Fig. 3. Effects of ranolazine (5, 10 and 20 μM) and DCA (1 mM) on the H$_2$O$_2$-induced changes in LVDP. The changes of LVDP in the vehicle (○), ranolazine (5 μM) (●), ranolazine (10 μM) (△), ranolazine (20 μM) (▲) and DCA (■) groups are shown. Values were calculated from the values of LVSP and LVEDP in Fig. 2. Each value represents a mean±S.E.M. *P<0.05, when compared with the value in the vehicle group.

Fig. 4. Effects of ranolazine (5, 10 and 20 μM) and DCA (1 mM) on the H$_2$O$_2$-induced changes in CPP. The changes of CPP in the vehicle (○), ranolazine (5 μM) (●), ranolazine (10 μM) (△), ranolazine (20 μM) (▲) and DCA (■) groups are shown. Hearts were those in Figs. 1 and 2. Each value represents a mean±S.E.M. *P<0.05, when compared with the value in the vehicle group.
group, respectively. Nevertheless, DCA (1 mM) did not attenuate the H$_2$O$_2$-induced decrease in LVDP. These results indicate that ranolazine attenuates the mechanical dysfunction induced by H$_2$O$_2$, whereas DCA hardly attenuates it.

Figure 4 shows the effects of ranolazine and DCA on the H$_2$O$_2$-induced changes in CPP. Before starting the H$_2$O$_2$ infusion, there was no significant difference in the CPP value between the vehicle and ranolazine or DCA groups. H$_2$O$_2$ induced a transient and slight decrease in CPP followed by a continuous and marked increase (P<0.001 by ANOVA). The increase in CPP induced by H$_2$O$_2$ was attenuated by ranolazine (20 μM) (P<0.05 by ANOVA followed by Dunnett’s test); this beneficial effect of ranolazine (20 μM) was observed at 13 to 33 min after starting the ranolazine infusion (P<0.05). Nevertheless, ranolazine (5 or 10 μM) or DCA (1 mM) did not attenuate the H$_2$O$_2$-induced increase in CPP. These results indicate that ranolazine attenuates the H$_2$O$_2$-induced increase in CPP and hence coronary resistance (because the perfusion flow is fixed) in a concentration-dependent way.

**Effects of ranolazine on energy metabolism**

Figure 5 shows the tissue levels of ATP, ADP, AMP and CrP at the end of the experiment in the normal (H$_2$O$_2$-untreated) and H$_2$O$_2$-treated hearts. In the normal (H$_2$O$_2$-untreated) heart, the tissue levels of ATP, ADP, AMP and CrP were similar between the vehicle and each of the ranolazine groups. In the H$_2$O$_2$-treated heart, H$_2$O$_2$ decreased the tissue levels of ATP (P<0.001 by ANOVA) and ADP (P<0.001 by ANOVA) and increased the tissue level of AMP (P<0.001 by ANOVA), although it did not change the tissue level of CrP (P=0.145 by ANOVA). The H$_2$O$_2$-induced change in the tissue level of ATP, however, was attenuated significantly by ranolazine (10 and 20 μM), suggesting that ranolazine attenuates the metabolic derangements induced by H$_2$O$_2$. Nevertheless, a low concentration of ranolazine (5 μM) failed to attenuate the H$_2$O$_2$-induced changes in the tissue level of ATP. Ranolazine (10 and 20 μM) tended to attenuate the H$_2$O$_2$-induced increase in the tissue level of AMP, although the effect of ranolazine on the H$_2$O$_2$-induced changes in the tissue levels of ADP and AMP was statistically insignificant. On the other hand, DCA (1 mM) did not attenuate the H$_2$O$_2$-induced changes in the tissue levels of ATP, ADP and AMP.

**Effect of ranolazine on lipid peroxidation**

There is a possibility that the beneficial effects of ranolazine on the H$_2$O$_2$-induced derangements relate to the reduction of lipid peroxidation that could be increased by H$_2$O$_2$. To examine this possibility, the tissue

![Figure 5](image)

**Fig. 5.** Effects of ranolazine (5, 10 and 20 μM) and DCA (1 mM) on the H$_2$O$_2$-induced changes in the tissue levels of high-energy phosphates. The tissue levels of ATP, ADP, AMP and CrP at the end of experiment (38 min after the start of ranolazine, DCA or vehicle infusion) were measured in the normal (H$_2$O$_2$-untreated) (open column) and H$_2$O$_2$-treated (hatched column) hearts. Hearts in the H$_2$O$_2$-treated groups are those in Figs. 2 and 3. Each value represents a mean±S.E.M. *P<0.05, when compared with the value in the corresponding normal group. †P<0.05, when compared with the value in the vehicle group in the H$_2$O$_2$-treated heart.

**Table 1.** Effect of ranolazine on H$_2$O$_2$-induced changes of the tissue level of MDA

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Tissue MDA level (nmol/g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (H$_2$O$_2$-untreated)</td>
<td>6</td>
<td>11.9±1.9*</td>
</tr>
<tr>
<td>Vehicle</td>
<td>8</td>
<td>49.6±8.4</td>
</tr>
<tr>
<td>Ranolazine (20 μM)</td>
<td>9</td>
<td>43.8±6.1</td>
</tr>
</tbody>
</table>

Each value represents a mean±S.E.M. n represents the number of hearts. The tissue level of MDA was measured 10 min after the end of H$_2$O$_2$ infusion. The final concentrations of ranolazine and H$_2$O$_2$ in the perfusate were 20 and 600 μM, respectively. *P<0.05, when compared with the value in the vehicle group.
level of MDA was measured in the normal (H₂O₂-untreated), vehicle and ranolazine (20 μM) groups (Table 1). In our previous study (14), the tissue level of MDA, which had been increased by H₂O₂, decreased by prolongation of the period of heart perfusion after the end of H₂O₂ infusion because MDA can be washed out into the myocardial interstitial effluent (21). Therefore, we measured the tissue MDA level shortly (10 min) after the end of H₂O₂ infusion. The “10 min” corresponds to the time when H₂O₂ produced a marked increase in LVEDP and when the increase in LVEDP was inhibited by ranolazine. In the vehicle group, H₂O₂ increased the tissue MDA level by about 4.2 times the value in the normal group (P<0.05, compared with the normal group). There was no statistical difference in the tissue level of MDA between the vehicle and ranolazine (20 μM) groups, however. These results indicate that ranolazine does not reduce the lipid peroxidation induced by H₂O₂.

DISCUSSION

In the present study, we examined the effect of ranolazine, a novel anti-ischemic drug, on the mechanical and metabolic derangements induced by reactive oxygen species in the isolated perfused rat heart. The reasons we used H₂O₂ as a reactive oxygen species can be summarized as follows: First, H₂O₂ and its metabolite, hydroxyl radical, are considered important in the pathogenesis of myocardial damage induced by ischemia-reperfusion (10, 22). Second, because H₂O₂ penetrates the cell membrane and reaches the intracellular site (23), it may produce severe damage to the cell. Last, 600 μM of H₂O₂ can inflict damage on the heart to a degree similar to that induced by ischemia-reperfusion in terms of accumulation of lipid peroxides (14). In the present study, H₂O₂ produced mechanical dysfunction (as evidenced by an increase in LVEDP and a decrease in LVDP) and metabolic changes (as evidenced by a decrease in the tissue level of ATP). These alterations induced by H₂O₂ were significantly attenuated by ranolazine (10 and 20 μM), suggesting that ranolazine has a beneficial effect on the H₂O₂-induced mechanical and metabolic derangements. According to some investigators (3–5), the concentrations of ranolazine (10 and 20 μM) used in the present study are also effective in attenuating myocardial derangements induced by ischemia and reperfusion. The action of ranolazine to reduce the H₂O₂-induced changes, therefore, may contribute to its cardioprotective action against ischemia-reperfusion damage.

In agreement with previous reports (5), the concentration of ranolazine (5, 10 and 20 μM) used in the present study did not modify mechanical function in the normal (H₂O₂-untreated) heart. In addition, ranolazine would not change coronary flow, because the hearts were perfused at a constant flow rate. It is unlikely, therefore, that the protective effects of ranolazine on the H₂O₂-induced myocardial derangements are due to an increase in coronary flow and/or a decrease in energy consumption of the heart. In fact, ranolazine did not have any effect on the tissue levels of high-energy phosphates in the normal (H₂O₂-untreated) heart. These results suggest that the protective effect of ranolazine on the H₂O₂-induced derangements is not due to preservation of energy caused by a favorable action on hemodynamics (i.e., energy-sparing effect).

Some of the radical scavengers or antioxidants have been demonstrated to protect the myocardium against oxidative stress (11, 12). In fact, we have reported that in the isolated perfused rat heart, catalase (a H₂O₂ scavenger) (14) or propofol (an intravenous anesthetic with an antioxidant action) (13) attenuates the H₂O₂-induced mechanical and metabolic derangements and lipid peroxidation. To determine whether ranolazine has a radical scavenging or an antioxidant effect, the tissue level of MDA was measured in the presence or absence of H₂O₂. We measured MDA by HPLC instead of using thiobarbituric acid, because the thiobarbituric acid method might measure not only MDA but also other substances (24). The results of the present study demonstrated that ranolazine did not affect the H₂O₂-induced lipid peroxidation, suggesting that the protective effect of ranolazine on the H₂O₂-induced derangements is not due to a radical scavenging effect or an antioxidant effect.

Ranolazine has been demonstrated to stimulate glucose oxidation through activation of pyruvate dehydrogenase in the myocardium (5, 19). Recently, Clarke et al. (19) have demonstrated in the isolated perfused rat heart that activation of pyruvate dehydrogenase with ranolazine occurs only when fatty acid (such as palmitate) is present in the perfusion solution. Because we used the fatty acid-free perfusion solution (KHB buffer), there is a possibility that activation of pyruvate dehydrogenase does not occur, and therefore mechanisms other than the action on glucose metabolism contribute to the beneficial effect of ranolazine on the H₂O₂-induced derangements. To determine this possibility, we examined whether DCA, which activates pyruvate dehydrogenase via inhibition of pyruvate dehydrogenase kinase (17–19), attenuates the H₂O₂-induced derangements. In contrast to ranolazine, DCA (1 mM) can activate pyruvate dehydrogenase in the myocardium regardless of the presence or absence of fatty acid in the perfusion solution (19). The results in the present study indicated that DCA was less effective in attenuating the H₂O₂-induced derangements. Therefore, the action of ranolazine on glucose metabolism may not be the primary mechanism of the protective effect against
the H₂O₂-induced derangements. However, we did not examine the effects of ranolazine and DCA on the activity of pyruvate dehydrogenase in the H₂O₂-treated heart.

According to recent studies, the reactive oxygen species including H₂O₂ cause excessive Na⁺ entry through the Na⁺ channel (25, 26), leading to intracellular Ca²⁺ overload through the Na⁺-Ca²⁺ exchange system (27), and hence mechanical dysfunction and metabolic derangement (28). We have found that lidocaine (14) and dilazep (15), both of which have a blocking action on the Na⁺ channel, attenuate the H₂O₂-induced mechanical and metabolic derangements. These facts suggest that inhibition of the Na⁺ channel is effective in attenuating the H₂O₂-induced myocardial derangements. Nevertheless, there is no evidence to show that ranolazine has a blocking action on the Na⁺ channel in the myocardial cell. Further studies are needed to determine detailed mechanisms of the protective action of ranolazine on the H₂O₂-induced derangements.

Recent studies also have revealed that reactive oxygen species including H₂O₂ produce endothelial dysfunction in coronary artery, which is responsible for myocardial derangements (29, 30). In fact, H₂O₂ caused a continuous and marked increase in CPP (i.e., the increase in coronary resistance) in the heart. Interestingly, the H₂O₂-induced increase in CPP was significantly attenuated by ranolazine (20 µM). Therefore, the cardioprotective action of ranolazine against the H₂O₂-induced derangements may, at least in part, result from inhibition of the H₂O₂-induced endothelial dysfunction.

In conclusion, ranolazine attenuates both mechanical and metabolic derangements induced by H₂O₂ through unknown mechanisms. This action of ranolazine may contribute to its protective action on the myocardium from ischemia-reperfusion damage.

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