Cardioprotective Effect of Ischemic Preconditioning on Ischemia/Reperfusion Injury in Spontaneously Type 2 Diabetic Rat Hearts

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ABSTRACT

Objectives and Methods : Although ischemic heart disease is significantly more prevalent and more severe in patients with diabetes mellitus than in the nondiabetic population, experiments have shown both increased and decreased susceptibility to ischemic injury under different experimental conditions. To clarify the cardioprotective effect of ischemic preconditioning (IP) on ischemia/reperfusion injury in spontaneously type 2 diabetic rats, we evaluated the duration of ischemia/reperfusion ventricular tachyarrhythmias (IRVT) using isolated rat hearts. We employed Otsuka Long-Evans Tokushima Fatty (OLETF) rats with obesity and low insulin sensitivity as the model for type 2 diabetes. After 5 minutes of aerobic perfusion, the rats were divided into the following groups : 1) control non–IP-treated rats (CIP−), 2) control IP-treated rats (CIP+), 3) diabetic non–IP-treated rats (DIP−), 4) diabetic IP-treated rats (DIP+). The IP protocol has three 2-minute cycles of ischemia followed by 5 minutes of reperfusion and 10 minutes of sustained ischemia.

Results : The duration of IRVT was significantly shorter in CIP+ (6.7±4.6 minutes) than in CIP− (17.7±2.0 minutes, $p<0.05$). There was, however, the duration of IRVT did not differ significantly between DIP− (16.5±3.6 minutes) and DIP+ (13.3±4.6 minutes). The degree of recovery of left ventricular function (left ventricular pressure, +dp/dt, −dp/dt, and coronary flow volume) after reperfusion was also significantly greater in CIP+ than in CIP−. However, the degree of recovery of left ventricular function did not differ significantly between DIP− and DIP+.

Conclusion : These results suggest that the cardioprotective effects of IP are attenuated in type 2 diabetic model rats. (Jikeikai Med J 2006 ; 53 : 69–79)

Key words : spontaneously type 2 diabetic rats, ischemic preconditioning, ischemia/reperfusion injury, ischemia/reperfusion ventricular tachyarrhythmias, recovery of cardiac function

INTRODUCTION

Ischemic heart disease is significantly more prevalent and more severe in patients with diabetes mellitus than in the nondiabetic population[1–3]. However, some animal studies suggest that diabetic hearts have greater resistance to ischemia, although other studies suggest they have lower resistance[4–7]. To date, no conclusive data have been obtained[8,9].

On the other hand, a preceding brief episode of myocardial ischemia increases ischemic tolerance and prevents myocardial dysfunction caused by subsequent sustained ischemic insult. This phenomenon is called ischemic preconditioning (IP)[10]. The cardioprotective effect of IP, however, has yet to be shown[11] experimentally in diabetic animal models. One rea-

son for this lack of confirmation is that the diabetes in animal models are usually induced with drugs, particularly streptzotocin.

Animals with streptzotocin-induced diabetes are emaciated and marked hyperglycemic due to a complete insulin deficiency from destruction of the pancreatic Langerhans cells, a pathological condition similar to that seen in type 1 diabetes mellitus. However, ischemic heart disease also develops in many patients who have type 2 diabetic with obesity or hyperlipidemia.

No consensus has been established regarding the ischemic tolerance observed in the hearts of animals with drug-induced diabetes or the cardioprotective effects obtained from IP. One possible reason for this lack of consensus is that the pathological conditions in animal models of drug-induced diabetes differ from those in patients with diabetes in whom ischemic heart disease develops. Therefore, clinically important data might be obtained from experiments in animal models of spontaneous type 2 diabetes that are similar to pathological conditions in humans. However, few studies of myocardial ischemia/reperfusion–induced myocardial injury and IP have been performed in animal models of spontaneous type 2 diabetes.

We hypothesized that IP-induced cardioprotection attenuates or disappears in the hearts of both type 2 diabetic model rats and type 2 diabetic human patients. In this study we tested this hypothesis by using the frequency and duration of ischemia/reperfusion-induced ventricular arrhythmia as indicators of IP.

METHODS

All experimental procedures and protocols used in this study were reviewed and approved by the animal experiment committee of The Jikei University School of Medicine.

Experimental animals

Otsuka Long-Evans Tokushima Fatty (OLETF) rats[14] were used as a model of spontaneous type 2 diabetes with insulin resistance, hyperlipidemia, and obesity. Long-Evans Tokushima Otsuka (LETO)

rats, which are genetically homologous to OLETF rats but do not have diabetes mellitus, were used as a nondiabetic control group. Rats in both groups were 30 weeks old when they were used for our experiment.

Apparatus used for perfusion and to induce ischemia

Each rat was anesthetized with pentobarbital (50 mg/kg) intraperitoneally, and then the heart was promptly removed and temporarily soaked in a cooled perfusion buffer to stop it from beating. A cannula was inserted into the aorta and used to perfuse it with 80 cm H_2O under constant pressure using the Langendorff method. Perfusion was subsequently performed using the working heart method[15] with the preload set to 10 cm H_2O and the afterload set to 80 cm H_2O. Modified Krebs-Henseleit bicarbonate buffer (pH 7.4) was used as the perfusion buffer. A one-way ball valve in aortic cannula was used to block coronary perfusion during diastole to induce ischemia. Electrical pacing (300 beats per minute, 3 V) was performed during ischemia.

Measurements of ischemia/reperfusion ventricular tachyarrhythmias and hemodynamics

During the experiment, electrocardiography was performed through carbon leads affixed to the surface of the heart. The incidence and duration of ischemia/reperfusion-induced ventricular tachyarrhythmias (IRVT), such as ventricular tachycardia and ventricular fibrillation that occurred during reperfusion, were recorded. Arrhythmias were analyzed according to the Lambeth Convention Criteria[17]. An 18-gauge catheter was inserted into the left ventricle through the left atrium to measure left ventricular pressure (LVP), LV max+dP/dt (LV+dP/dt), LV max−dP/dt (LV−dP/dt) using a polygraphic system (Fukuda Electron, Tokyo).

Measurement of lactate and H^+ in the coronary effluent

To avoid contact with air, the coronary effluent samples were taken through a cannula inserted into the pulmonary artery. PO_2, PCO_2, HCO_3^-, pH were measured using a blood gas analyzer (Corning 175, COMPANY, CITY, STATE, USA). H^+ was calcu-

lated with values of PCO_2 and HCO_3^- according to the following formula.

$$[H^+] (nmol/l) = 24 \times PCO_2 (mmHg) / [HCO_3^-]$$
$$(nmol/l).$$

Experimental protocol

The working hearts of rats were perfused for 5 minutes under constant pressure. The rats were then divided into the following 4 groups: 1) nondiabetic control rats not undergoing IP (CIP−), 2) nondiabetic control rats undergoing IP (CIP+), 3) diabetic rats not undergoing IP (DIP−), and 4) diabetic rats undergoing IP (DIP+). The rats not undergoing IP were perfused under constant pressure for 21 minutes and kept in an ischemic condition for 10 minutes. The rats with IP were kept in an ischemic condition for two minutes and reperfused for 56 minutes; this cycle was repeated 3 times, after which an ischemic condition was maintained for 10 minutes. After this procedure, all rats were again perfused for 20 minutes (Fig. 1).

Statistical analysis

When comparing the two groups for equality of variances, Student's t-test was used, and when comparing the two groups for inequality of variances, the Cochran test was used. Fisher's exact test was used to compare the frequencies of IRVT. All data are expressed as means±standard error, and $p < 0.05$ indicated statistical significance.

RESULTS

Body weight and glycosylated hemoglobin

Body weight and levels of glycosylated hemoglobin (HbA_{1c}) were significantly higher in the diabetic groups than in the nondiabetic control groups (Fig. 2). Serum insulin levels were slightly but not significantly higher in the diabetic groups than in the nondiabetic groups.

Frequency of IRVT

The frequency of IRVT was slightly but not significantly higher in the CIP− group (100%, 5 of 5 rats) than in CIP+ group (33%, 2 of 6 rats, $p = 0.061$; Fig. 3A, 3B) and did not differ between the DIP− group (83%, 5 of 6 rats) and the DIP+ group (67%, 4 of 6 rats, Fig. 3D, 3E).

Mean duration of IRVT

The mean duration of the IRVT was significantly shorter in CIP+ (17.7±2.0 minutes) than in CIP− group (6.7±4.6 minutes, $p < 0.05$; Fig. 3C). In contrast, the duration of IRVT did not differ signficantly between the DIP− group (16.5±3.6 minutes) and the
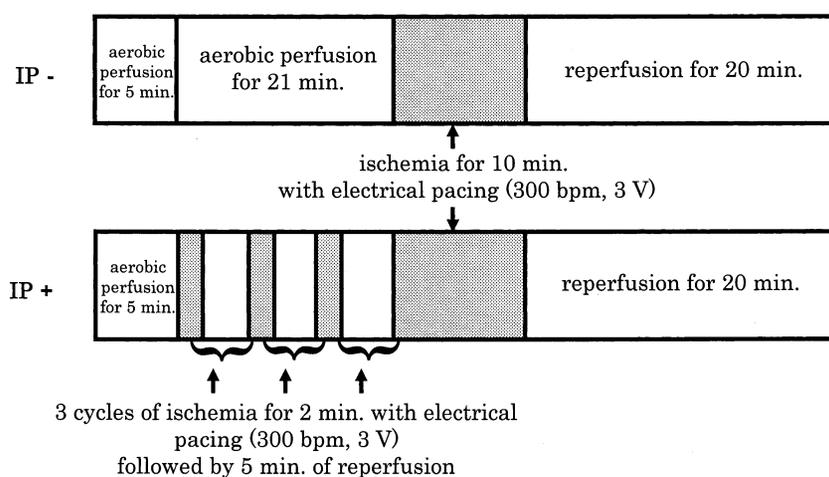

Fig. 1. Perfusion protocols. Protocol 1: After 5 minutes of aerobic perfusion, IP− received an additional 21 minutes of aerobic perfusion followed by 10 minutes of ischemia with electrical pacing (300 bpm, 3V). Protocol 2: The IP+ group received three cycles of ischemia for 2 minutes followed by 5 minutes of reperfusion with electrical pacing (300 bpm, 3V), followed by 10 minutes of ischemia. Both protocols included an additional 20 minutes of reperfusion.

DIP+ group (13.3±4.6 minutes, Fig. 3F).

Recovery of left ventricular pressure after reperfusion

The degree of recovery of left ventricular pressure 1 and 2 minutes after reperfusion was significantly greater in the CIP+ group than in the CIP− group (Fig. 4A). In contrast, the degree of recovery of left ventricular pressure after reperfusion did not differ significantly between the DIP− group and DIP+ group (Fig. 4B).

Recovery of coronary flow after reperfusion

The degree of recovery of coronary flow 1 and 2 minutes after reperfusion was significantly greater in
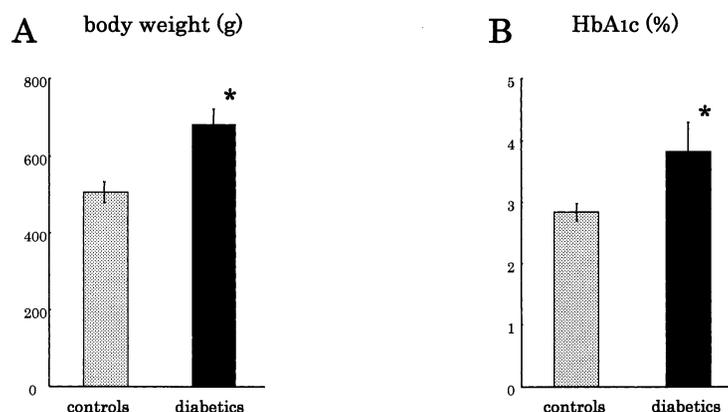

Fig. 2. Comparison of body weights and HbA1C values for control rats and diabetic rats.
Diabetic rats had significantly higher body weights and HbA1C levels than did control rats. Data are shown as mean±SEM. *$p < 0.01$ compared with control
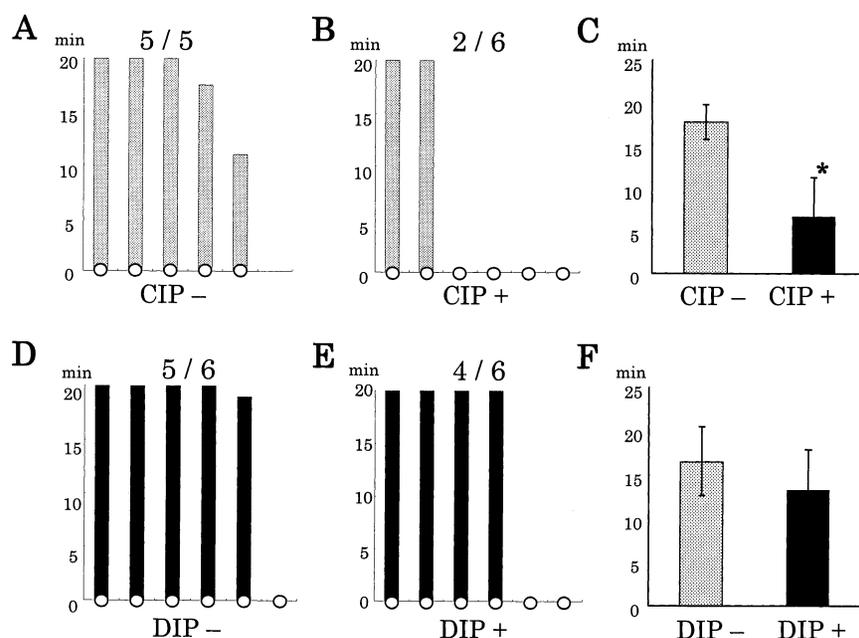

Fig. 3. Incidence and mean duration of IRVT in control rats and diabetic rats.
Incidence of IRVT: 100% (5 of 5) in CIP− (Fig. 3A); 33% (2 of 6) in CIP+ (Fig. 3B); 83% (5 of 6) in DIP− (Fig. 3D); and 67% (4 of 6) in DIP+ (Fig. 3E). CIP− tended to have a higher incidence of IRVT than did CIP+. There was no significant difference in the incidences of IRVT in DIP− and DIP+. The mean duration of IRVT was significantly shorter in CIP+ than in CIP− (Fig. 3C). The duration of IRVT did not differ significantly between DIP− and DIP+ (Fig. 3F). Data are shown as mean± SEM. *$p < 0.05$ compared with IP−

the CIP+ group than in the CIP− group (Fig. 5A). Coronary flow 2 minutes after reperfusion was significantly greater in the DIP+ group than in the DIP+ group (Fig. 5B).
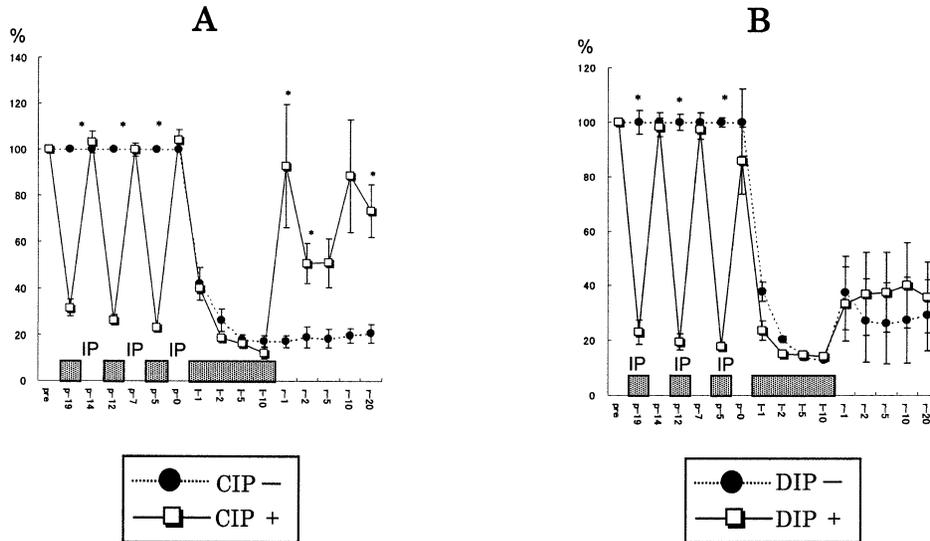

Fig. 4. Time course of changes in LV pressure recovery in control rats and diabetic rats.
A : LV pressure recovery between CIP− and CIP+. B : LV pressure recovery between DIP− and DIP+. The extent of LV pressure recovery at 1, 2, and 20 minutes after reperfusion was significantly greater in CIP+ than in CIP−. The extent of LV pressure recovery after reperfusion did not differ between DIP− and DIP+. Data are shown as mean±SEM. *$p < 0.05$ compared to IP−.
p : before 10 minutes of ischemia, I : during 10 minutes of ischemia, r : after 10 minutes of ischemia, Numbers mean time (min).
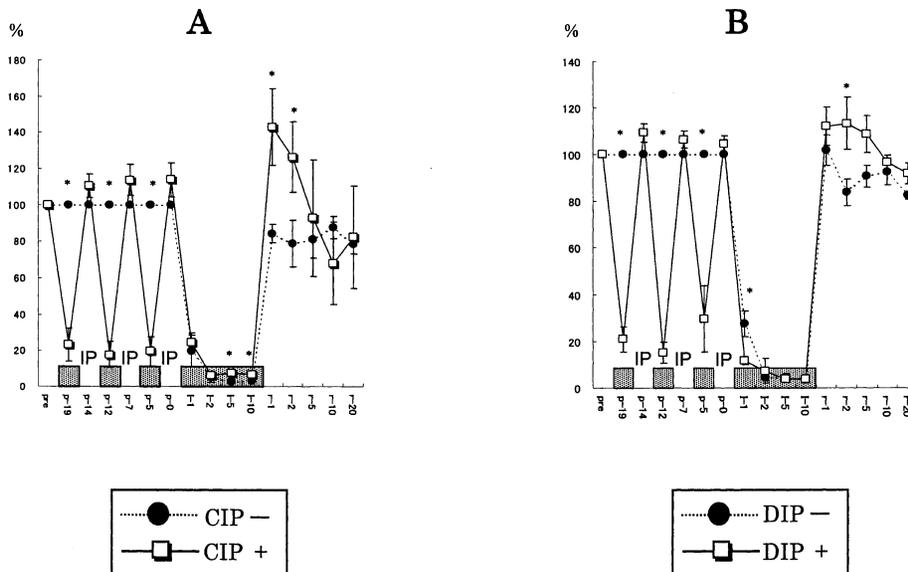

Fig. 5. Time course of changes in coronary flow recovery in control rats and diabetic rats.
A : Coronary flow recovery between CIP− and CIP+. B : Coronary flow recovery between DIP− and DIP+. Coronary flow at 1 and 2 minutes after reperfusion was significantly greater in CIP+ than in CIP−. Coronary flow in DIP+ 2 minutes after reperfusion was significantly greater than that in DIP−. Data are shown as mean±SEM. *$p < 0.05$ compared to IP−.
p : before 10 minutes of ischemia, I : during 10 minutes of ischemia, r : after 10 minutes of ischemia, Numbers mean time (min).
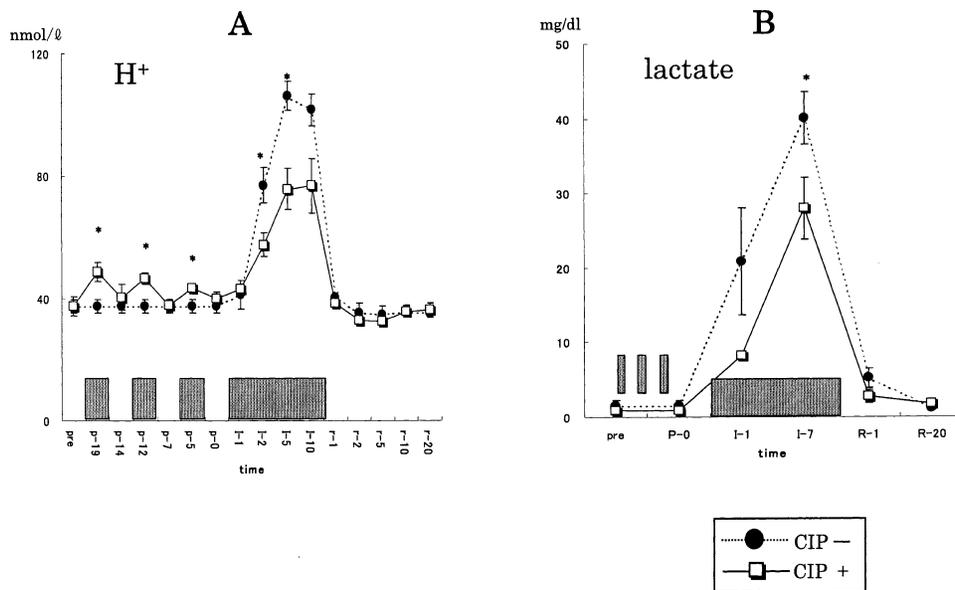
Fig. 6. Time course of changes in H^+ and lactate levels of diabetic rats with and without IP during 10 minutes of ischemia.

H^+ and lactate levels during 10 minutes of ischemia were significantly attenuated in CIP$+$ as compared with those in CIP$-$. Data are shown as mean$\pm$SEM. *$p<0.05$ compared with IP$-$.

p: before 10 minutes of ischemia, I: during 10 minutes of ischemia, r: after 10 minutes of ischemia, Numbers mean time (min).

Fig. 7. Time course of changes in H^+ and lactate levels of diabetic rats with and without IP during 10 minutes of ischemia.

H^+ and lactate levels during 10 minutes of ischemia were significantly attenuated in DIP$+$ as compared with those in DIP$-$. Data are shown as mean$\pm$SEM. *$p<0.05$ compared with IP$-$.

p: before 10 minutes of ischemia, I: during 10 minutes of ischemia, r: after 10 minutes of ischemia, Numbers mean time (min).
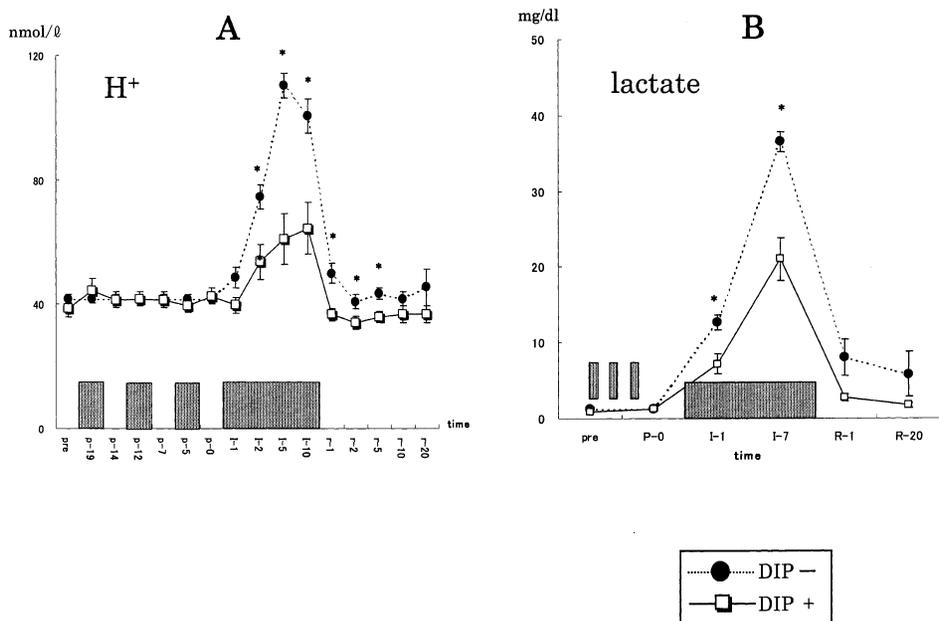
Recovery of $+dp/dt$ and $-dp/dt$ after reperfusion

The degree of recovery $+dp/dt$ and $-dp/dt$ after reperfusion was significantly greater in non-diabetic control rats undergoing IP than in those not undergoing IP.

However, the degree of recovery of $+dp/dt$ and $-dp/dt$ did not differ significantly between diabetic rats undergoing IP and those not undergoing IP (data not shown).

Changes of lactate levels and $H+$ levels in coronary effluent during 10-minute ischemia.

Lactate levels in coronary effluent after 7 minutes of the 10-minute ischemia period were significantly greater in the CIP— group than in the CIP+ group. Lactate levels in the coronary effluent after 1 and 7 minutes after the start of ischemia were significantly greater in the DIP— group than in the DIP+ group (right panels in Fig. 6, 7). The H^+ levels were significantly greater in the CIP— group than in the CIP+ group and in the DIP— group than in the DIP+ group (left panels of Fig. 6, 7).

DISCUSSION

Usefulness of OLETF rats as an animal model of spontaneous type 2 diabetes

The OLETF rats used in this study as an animal model of type 2 diabetes were produced by selective breeding of rats from the Long-Evans strain and are hyperglycemic, as shown by serum glucose levels of 300 mg/dl or greater 60 minutes after oral glucose loading. Diabetes mellitus develops in these rats after the age of 24 weeks along with obesity, hypertriglyceridemia, and hyperinsulinemia due to low insulin sensitivity. Insulin levels decrease after the age of 65 weeks[14]. In contrast, rats with streptozotocin-induced diabetes have lean bodies and serum ketoacidosis due to lack of insulin secretion. These rats are a model of type 1 diabetes.

We used 30-week-old OLETF rats with HbA1C levels and body weights significantly greater than those in age-matched LETO rats. Serum insulin levels also tended to be higher in OLETF rats. The pathological features of OLETF rats resemble those of humans with obesity and type 2 diabetes, making OLETF rats a useful animal model for studying the pathologic changes of diabetes mellitus.

The difference in the effects of IP between patients with diabetes and experimental (spontaneously type 2 and drug induced) diabetic models Ishihara, et al.[21] have reported that patients without diabetes mellitus who have angina pectoris before a first myocardial infarction experience little or no loss of cardiac function and have a lower in-hospital mortality rate. This finding suggests that the IP effects of angina pectoris before myocardial infarction contribute to cardioprotection in patients without diabetes. On the other hand, the finding that patients with diabetes mellitus showed no difference in cardiac function or in-hospital mortality rate regardless of the presence of angina pectoris before a first myocardial infarction suggests that the IP effect had disappeared. Jimenez-Navarro, et al.[22] have found that in patients with diabetes mellitus cardiac function in the acute phase after myocardial infarction is not affected by the presence of angina pectoris before myocardial infarction. On the other hand, Kristiansen, et al.[18] have reported that IP does not have cardioprotective effects on either an extensive myocardial infarction area or the recovery of cardiac function after reperfusion in rats with spontaneous type 2 diabetes.

In this study using a rat model of type 2 diabetes, the effects of IP on ventricular arrhythmia after reperfusion were attenuated, a finding that does not conflict with previous findings in this animal model.

However, experiments using models of drug-induced diabetes have yielded varying results. A study by Tosaki, et al.[19] using rats with streptozotocin-induced diabetes found that IP does not exert an antiarrhythmic effect. In contrast, Ravingerova, et al.[20] have found that IP suppresses arrhythmia 1 week after intravenous injection of streptozotocin to induce diabetes but that the effect had disappeared by 9 weeks after injection. However, Tatsumi, et al.[9] have reported that IP facilitates recovery of left ventricular function in rats with streptozotocin-induced diabetes and have concluded that the diabetic myocardium may benefit from preconditioning-in-

duced cardioprotection.

As mentioned above, the effects of IP in rats with spontaneous type 2 diabetes tend to resemble those in patients with diabetes mellitus. The reported effects in rats with streptozotocin-induced diabetes, however, are not uniform, possibly because of differences in experimental conditions. Streptozotocin-induced diabetes resembles type 1 diabetes, which may explain the differences in the effects of IP from those in animals and human patients with spontaneous type 2 diabetes[34–36].

Mechanism of IP-induced cardioprotective effects in the experimental diabetic model hearts
Involvement of lactate and H+ in extended ischemia following IP

A study[9] that examined the recovery of left ventricular function after reperfusion using rats with streptozotocin-induced diabetes found that IP promotes the recovery of left ventricular function. This finding suggests that IP exerts cardioprotective effects in rats with streptozotocin-induced diabetes. The authors have also suggested that the mechanism underlying these effects is the suppression of anaerobic glycolysis during extended ischemia following IP. Diabetic rats store more glycogen in the myocardium than do normal rats. However, because glycogen levels in the diabetic rats decrease to levels similar to those in normal rats[9] following IP, diabetes rats and control rats showed no differences in the production of glycogen metabolites, including lactate and H^+, during sustained ischemia. Another study in rats with streptozotocin-induced diabetes[23] found that IP almost completely eliminates myocardial glycogen stores, which reduces the production of lactate metabolites and H^+ during sustained ischemia but has little effect on the suppression of cardiac function recovery after reperfusion. According to these two studies, the severity of ischemia/reperfusion injury observed in rats with streptozotocin-induced diabetes depends upon decreased levels of glycogen in the myocardium at the start of long-lasting ischemia following IP.

In the present study study, the production of lactate and H^+ during long-lasting ischemia was lower in both nondiabetic control rats and diabetic rats that underwent IP than in rats that did not undergo IP. In other words, the decreased production of lactate and H^+ during sustained ischemia following IP suppresses arrhythmia after reperfusion in the nondiabetic control rats but not in OLETF rats with spontaneous type 2 diabetes. Therefore, the suppression of arrhythmia in rats with spontaneous type 2 diabetes cannot be explained only by the attenuated levels of H^+ and lactate due to IP and suggest that additional factors are be involved in the mechanism.

Glucose metabolism and nitric oxide are reportedly involved in the mechanism of IP in patients with or without diabetes. Hyperglycemia is considered a major determinant of the extent of ischemia/reperfusion injury in experimental models of diabetes. On the other hand, a persistent hyperglycemic environment before ischemia/reperfusion may induce myocardial adaptation, which inhibits the effects of IP. Impaired nitric oxide metabolism in models of diabetes may play an important role in IP, but its involvement is unclear. Because the metabolic mechanism responsible for attenuated myocardial protection afforded by IP in models of type 2 diabetes remains unclear, further studies of metabolic changes in myocardium during ischemia/reperfusion should be performed.

Involvement of myocardial ion channels in normal control rats, OLETF diabetic rats, and rats with streptozotocin-induced diabetes.

In normal control rats anaerobic glycolysis-induced H^+ production activates the Na^+/H^+ exchanger (NHE) and the Na^+/HCO_3^- co-transport system of cardiomyocytes. This activation promotes Na^+ cellular inflow, which causes intracellular Na^+ ($[Na^+]_i$) storage. The Na^+/Ca^{2+} exchanger uses this stored Na^+ to suppress Ca^{2+} efflux from cardiomyocytes, which results in the storage of intracellular Ca^{2+} ($[Ca^{2+}]_i$) in cardiomyocytes. With the decrease in acidosis after reperfusion, Na^+/Ca^{2+} exchanger activity, which has been suppressed, accelerates, and reverses its action to generate $[Ca^{2+}]_i$ overload[24]. This $[Ca^{2+}]_i$ overload causes the car-

diomyocytes to contract excessively, rupturing the myocardial membrane and leading to cell necrosis. Thus, in normal control rats changes in the activation of ion channels during ischemia/reperfusion play a critical role in myocardial injury.

No previous reports have analyzed changes in the activation of ion channels of cardiomyocytes in OLETF rats. Because the ability to secrete insulin might be retained or accelerated in this animal model, the NHE activity is not thought to be significantly lower than in normal rats. Moreover the reduction in $[Ca^{2+}]_i$ due to functional changes of the cellular membrane does not suppress NHE activity, suggesting that, in type 2 diabetic model rats, NHE activity is generally preserved or promoted if insulin secretion is maintained or increased. This condition may exacerbate ischemia/reperfusion-induced myocardial injury and suppress IP-induced cardioprotection in OLETF rats.

On the other hand, suppression of NHE activity due to insulin depletion is observed in rats with streptozotocin-induced diabetes[25-27], and the possibility cannot be ruled out that this suppressed activity inhibits $[Ca^{2+}]_i$ overloading and prevents ischemia/reperfusion-induced myocardial injury.

Results of an experiment using rats with streptozotocin-induced diabetes suggest that a decreased $[Ca^{2+}]_i$ due to functional changes in the cellular membrane plays a role in decreasing NHE activity[28]. Another study[29] has shown that in experimentally produced type 1 diabetic hearts, the transmembrane Na^+ gradient decreases because of an increase in $[Na^+]_i$, which may suppress NHE activity. Unlike in OLETF rats, this suppressed activity in rats with streptozotocin-induced diabetes may prevent $[Ca^{2+}]_i$ overload, inhibit ischemia/reperfusion injury, and preserve the effects of IP.

Involvement of the ATP-sensitive potassium channels in the cardiomyocytes

The ATP-sensitive potassium channels (KATP) are activated when intracellular ATP decreases due to myocardial ischemia, and the outward K^+ current shortens the action potential duration of the cardiomyocyte. As a result, $[Ca^{2+}]_i$ decreases, and the myocardial damage caused by the $[Ca^{2+}]_i$ overload lessens[30]. The opening of the KATP channels in the myocardium is one mechanism of IP-induced cardioprotection[21]. The KATP channels in the cell membrane and in mitochondria may both be involved in this mechanism[31,32].

Smith[33] has reported that KATP channels in the myocytes in streptozotocin-induced diabetic rats are more sensitive and open at higher intracellular ATP levels than do KATP channels in normal control rats. Chronic hypoxia in rats with streptozotocin-induced diabetes modifies the gene expression of the myocardial KATP channels and alters all channel activity by affecting the volume expressed in the channels, which causes resistance to ischemia/reperfusion-induced arrhythmia[37,38].

However, no study has examined the relationship between the myocardial KATP channels and the cardioprotection in rats with spontaneous type 2 diabetes. The threshold for opening the myocardial KATP channels in rats with type 2 diabetes may differ from that in rats with drug-induced diabetes. The threshold for opening KATP channels in rats with spontaneous type 2 diabetes is significantly higher than that in rats with drug-induced diabetes, a finding that suggests KATP channels do not open in rats with spontaneous type 2 diabetes unless the myocardial ATP level falls below a threshold level that opens KATP channels in rats with drug-induced diabetes. In other words, the myocardial ATP level that opens KATP channels in rats with spontaneous type 2 diabetes may be lower than that in normal control rats. The underlying mechanism for the altered activity of KATP channels is unclear. Low insulin sensitivity and acidosis in rats with spontaneous type 2 diabetes may modify the gene expression of channels and alter the global activity of the KATP channel.

No experiments using OLETF rats with spontaneous type 2 diabetes have examined the sensitivity of KATP channels. Future detailed study should include measuring the threshold of channel opening with the patch-clamp technique and observing the degree of suppression of IP-induced cardioprotection by drugs that block KATP channels.

Limitations of this study

In this study, we simultaneously measured the duration of IRVT and the recovery of cardiac function after reperfusion as indicators of IP–induced cardioprotection. Our experiment demonstrated a close relationship between IRVT and cardiac function recovery, suggesting that IRVT greatly affects left ventricular function. When IRVT occurs, effective cardiac contraction disappears and left ventricular pressure cannot be measured. Both the duration of IRVT and cardiac function cannot be examined simultaneously. Therefore, to precisely examine the recovery of cardiac function, the outcomes of IRVT after electrocardioconversion has re–established sinus rhythm must be compared or another experiment protocol that assumes little or no arrhythmia must be used.

CONCLUSIONS

We examined IP–induced cardioprotection using OLETF rats with spontaneous type 2 diabetes from which the hearts were isolated and perfused. IP–induced suppression of IRVT and cardiac function recovery after reperfusion were observed in normal control LETO rats but not in OLETF rats. These results prove our hypothesis that IP–induced cardioprotection is attenuated or eliminated in rats with spontaneous type 2 diabetes. These results are similar to clinically reported pathological phenomena.

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