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Myocardial Susceptibility to Ischemia and the Effect of Ischemic Preconditioning in Rats with Streptozotocin-induced Diabetes

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ABSTRACT

Objective and Method : To investigate myocardial susceptibility to ischemia and the cardioprotective effects of ischemic preconditioning (IP) on ischemia/reperfusion injury, we evaluated reperfusion ventricular tachyarrhythmias (rVT) using the isolated hearts of rats with streptozotocin-induced diabetes. After 5 minutes of initial aerobic perfusion, both the control rats and the diabetic rats were divided into a group that underwent IP (IP+) and a group that did not IP (IP−). The IP protocol called for 3 cycles of ischemia (2 minutes) followed by reperfusion (5 minutes) before a long period of ischemia. The incidence and duration of rVT were recorded as markers of susceptibility to ischemia and the effects of IP.

Results : The control rats in the IP+ and IP− groups showed no significant difference in the incidence of rVT (100% vs. 60%). However, the duration of rVT was significantly shorter in the IP+ group (6.7±5.2 minutes) than in the IP− group (15.5±4.4 minutes, $p<0.05$). Both the incidence and duration of rVT were less in the diabetic IP+ group (0% and 0 minutes) than in the diabetic IP− group (100%, $p<0.05$, and 6.5±2.1 minutes, $p<0.05$). Furthermore, the diabetic IP− group had significantly shorter rVT durations than did the control IP− group (6.5±2.1 min. vs. 15.5±4.4 min, $p<0.05$).

Conclusion : These results suggest that rats with streptozotocin-induced diabetes are less susceptible to ischemia/reperfusion injury than are normal control rats and that the cardioprotective effects of IP are preserved in rats with streptozotocin-induced diabetes.

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Key words : rats with streptzotocin-induced diabetes, ischemia/reperfusion injury, ischemic preconditioning, cardioprotective effect

INTRODUCTION

Diabetes mellitus is a major risk factor for ischemic heart diseases and is easily aggravated[1-3]. In particular, the incidence of ischemic heart diseases is increased in patients with type 2 diabetes complicated by obesity or hyperlipidemia. Approximately half of the experiments using cardiomyocytes from rats with streptozocin-induced diabetes have shown increased susceptibility to ischemia, whereas the remaining half report conflicting results or decreased susceptibility to ischemia[4-7]. No consensus has been reached on this issue[8,9]. On the other hand, a short period of myocardial ischemia increases susceptibility to ischemia and acts as ischemic preconditioning (IP) to protect against myocardial injury from a subsequent long period of ischemia[10]. No conclusive data have been obtained regarding the cardioprotective effects

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of IP in rats with streptozocin-induced diabetes[11]. Such rats do not secrete insulin in the required volumes to maintain normoglycemia and have markedly increased blood glucose levels, emaciation, and ketoacidosis similar to those seen in type 1 diabetes mellitus. On the other hand, ischemic heart diseases develop more often in patients with type 2 diabetes mellitus complicated by obesity, insulin resistance, and hyperlipidemia. This presentation does not correspond to that of the rat model of drug-induced diabetes.

In this study, we used isolated, perfused hearts from rats with streptozocin-induced diabetes to examine changes in the susceptibility of cardiomyocytes to ischemia and to examine the cardioprotective effects of IP. The incidence and duration of reperfusion ventricular arrhythmia (rVT) were used as indices for evaluation.

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 animal models

Male Sprague-Dawley rats were divided into 2 groups. The first group consisted of control rats, which received injections of physiological saline through a tail vein at 10 weeks of age ; 10 weeks later, their hearts were removed. The second group consisted of diabetic animal model rats. Streptozocin was dissolved in a 50 mM citrate buffer (pH 4.8) and injected into a tail vein ; 10 weeks later, the rats' hearts were removed. Diabetic rats with a fasting blood glucose (FBS) of more than 400 mg/dl were used in this study. In addition, levels of FBS and glycosylated hemoglobin (HbA_{1C}) were measured with blood samples obtained from the inferior vena cava just before the experiment after 18 hours of fasting. The FBS level was measured with the glucose oxidase method[12], and the HbA1C level was measured with the latex method[13].

Apparatus used for perfusion and production of ischemia

Each rat was anesthetized intraperitoneally with 50 mg/kg of pentobarbital. The heart was then promptly removed and temporarily immersed in a cooled, perfusion buffer in to prevent contractions. Next, a cannula was inserted into the aorta and used to perfuse the aorta with modified Krebs-Henseleit bicarbonate buffer (pH 7.4) at a constant pressure of 80 cm H_2O using the Langendorff method. Perfusion was subsequently performed with the working heart method[14], with the preload set to 10 cm H_2O and the afterload set to 80 cm H_2O. A one-way ball valve in the cannula was inserted into the aorta to block coronary perfusion during diastole and induce ischemia. Electrical pacing (300 bpm, 3 V) was performed during ischemia.

Measuring ischemia/reperfusion rVT and hemodynamics

Electrocardiograms were recorded during the experiment via carbon leads affixed to the surface of the heart. Also recorded were the incidence and duration of ischemia/reperfusion-induced rVTs, such as ventricular tachycardia and ventricular fibrillation, during reperfusion. Arrhythmias were analyzed according to the Lambeth Convention Criteria[16]. An 18-gauge catheter inserted into the left ventricle through the left atrium was used with a polygraphic system (Fukuda Electron, Tokyo, Japan) to measure left ventricular pressure : LV_{max} +dP/dt (LV+dP/dt), LV_{max} −dP/dt (LV−dP/dt).

Measuring lactate and H^+ in the coronary effluent

To avoid contact with air, the coronary effluent samples were obtained over time through a cannula inserted into the pulmonary artery. The PO_2, PCO_2, HCO_{3-}, and pH were measured with a blood gas analyzer (Corning 175, New York, USA). H^+ was calculated with values of PCO_2 and HCO_{3-} according to the following formula.

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

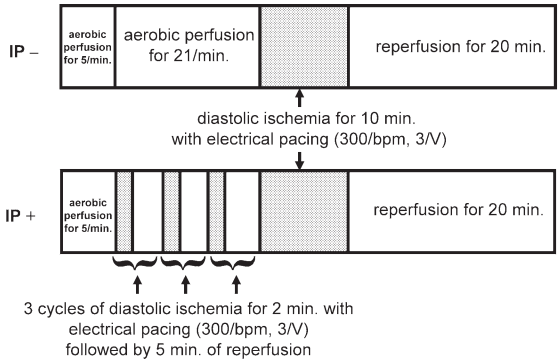

Fig. 1. Perfusion protocols. Protocol 1: After 5 minutes of aerobic perfusion, the IP− group received an additional 21 minutes of aerobic perfusion followed by 10 minutes of ischemia with electrical pacing (300 bpm, 3 V). Protocol 2: The IP+ group received 3 cycles of ischemia for 2 minutes followed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

Experimental protocol

The working hearts of the rats were perfused for 5 minutes under constant pressure. The rats were then divided into following 4 groups: 1) nondiabetic control rats not undergoing IP (IP−), 2) nondiabetic control rats undergoing IP (IP+), 3) diabetic IP− rats, and 4) diabetic IP+ rats. The IP− rats were perfused under constant pressure for 21 minutes, after which ischemic conditions were maintained for 10 minutes. For IP+ rats, 3 sets of 2-minute periods of ischemia and a subsequent 5-minute period of reperfusion were applied, after which ischemia was induced for 10 minutes. For both IP+ and IP− rats, ischemia was induced for 10 minutes, and reperfusion was performed for 20 minutes (Fig. 1).

Statistical analysis

Groups were compared using Student's t-test for equality of variances and the Cochran-Cox test for inequality of variances. Fisher's exact test was used to compare the frequencies of rVT. All data are expressed as means±standard error, and $p<0.05$ indicated statistical significance.

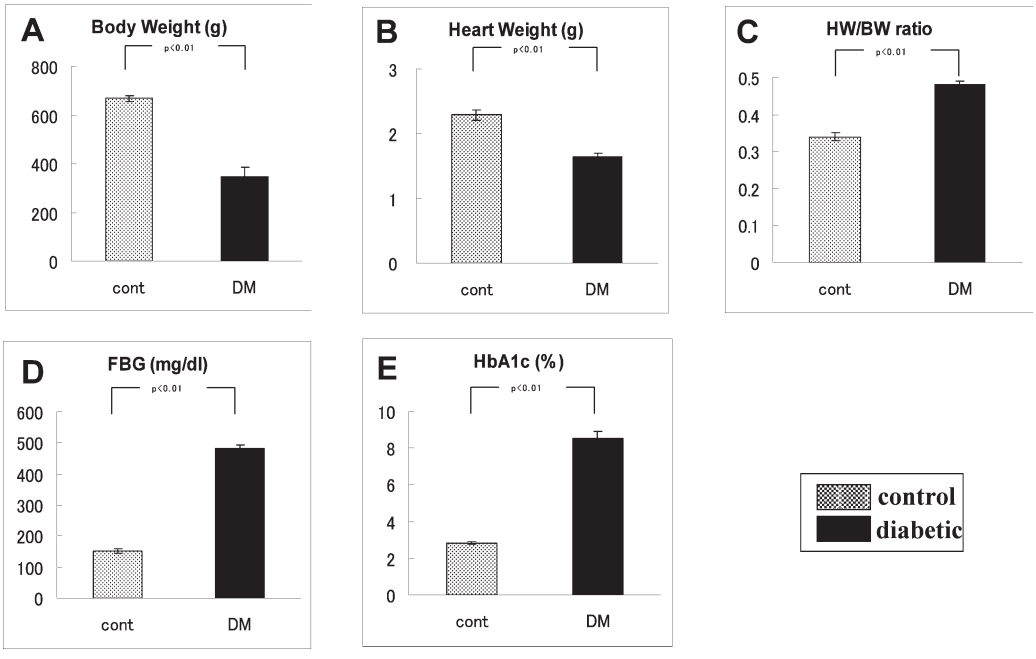

Fig. 2. Comparison of body weights and HbA_{1C} between control rats and diabetic rats. Diabetic rats had significantly lower body weights (A) and significantly higher levels of FBS (D) and HbA_{1C} (E) than did control rats. Diabetic rats had significantly lower heart weights than did control rats (B), but also had significantly higher heart weight/body weight ratios (C). Data are shown as means±SEM. *$p<0.01$ compared with control.

RESULTS

1. Body weight

Body weight was significantly lower in diabetic rats (347±38 g) than in control rats (668±39 g, $p<0.01$, Fig. 2A).

2. Heart weight

Heart weight was significantly lower in diabetic rats (1.65±0.18 g) than in control rats (2.29±0.29 g, $p<0.01$, Fig. 2B.

3. Heart weight/body weight ratio

Heart weight/body weight ratio of diabetic rats (0.48±0.04) was significantly higher than that of control rats (0.34±0.03, $p<0.01$, Fig. 2C).

4. FBS

FBS was significantly higher in diabetic rats (482±41 mg/dl) than in control rats (152±25 mg/dl, $p<0.01$, Fig. 2D).

5. HbA_{1C}

Levels of HbA_{1C} were significantly higher in diabetic rats (8.5±1.5%) than in control rats (2.8±0.2%, $p<0.01$, Fig. 2E).

6. Ischemia/reperfusion-induced rVTs

1) Frequency of rVT

No significant differences were observed in the frequency of rVT in control rats between the IP− (100%, 5 of 5 rats; Fig. 3A) and IP+ groups (60%, 3 of 5 rats; Fig. 3B). However, in diabetic rats, the frequency of rVT was significantly ($p<0.05$) lower in the IP+ group (0%, 0 of 5 rats; Fig. 3C) than in the IP− group (100%, 5 of 5 rats; Fig. 3D).

2) Mean duration of rVT

In control rats, the mean duration of rVT was significantly shorter in the IP+ group (6.7±5.2 minutes) than in the IP− group (15.5±4.4 minutes, $p<0.05$, Fig. 4A). In diabetic rats, the mean duration was significantly shorter in the IP+ group than in the IP− group (6.5±2.1 minutes. vs.., $p<0.05$) (Fig. 4B). The mean duration of rVT was significantly shorter in the diabetic IP− group (6.5±2.1 minutes) than in the control IP− group (15.5±4.4 minutes, $p<0.05$, Fig. 4A, B).

7. Changes in left ventricular function after reperfusion

1) Restoration of coronary flow after reperfusion

In control rats, coronary flow recovery after 20 minutes of reperfusion was significantly greater in the

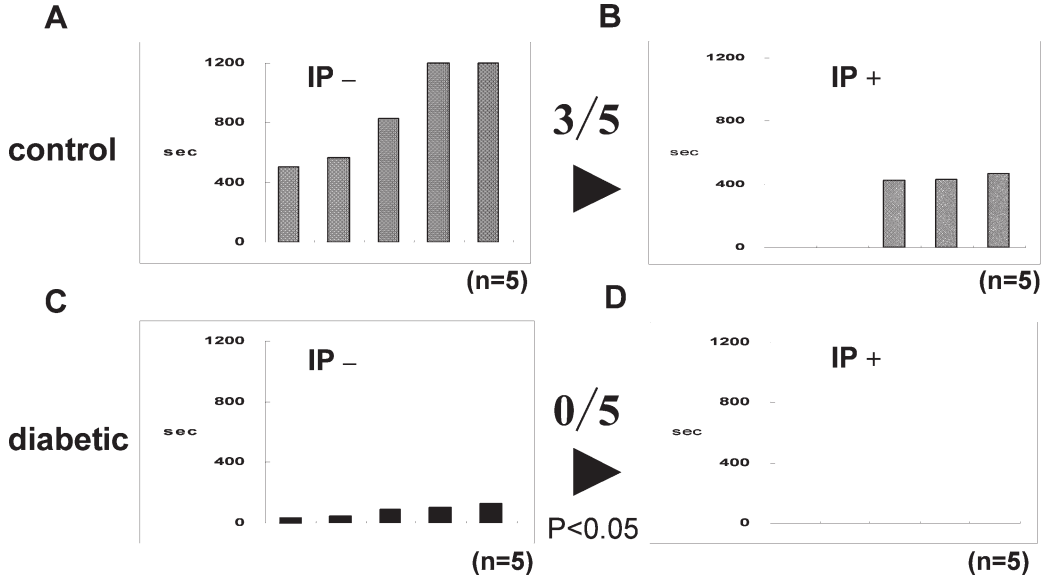

Fig. 3. Incidence of rVT in control rats and diabetic rats. There was no significant difference in the incidence of rVT in control IP+ rats (100%) and IP− rats (60%). However in diabetic rats, the rate of was significantly lower rVT in the IP+ group (0%) than in the IP− group (100%, $p<0.05$).

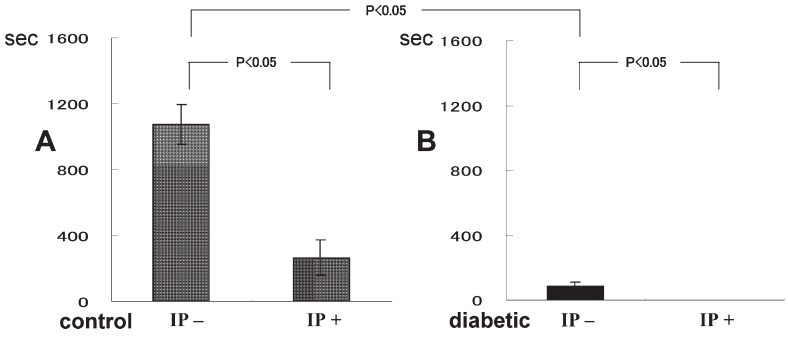

Fig. 4. Mean duration of rVT in control rats and diabetic rats ($n=5$ or 6).
A: The duration of rVT was significantly shorter in the control IP+ group (6.7±5.2 minutes) than in control IP− group (15.5±4.4 minutes, $p<0.05$).
B: The duration of rVT was also significantly shorter in the diabetic IP+ group (0 minutes) than in the diabetic IP− group (6.5±2.1 minutes, $p<0.05$). Data are shown as means±SEM. *$p<0.05$ compared with IP− group.

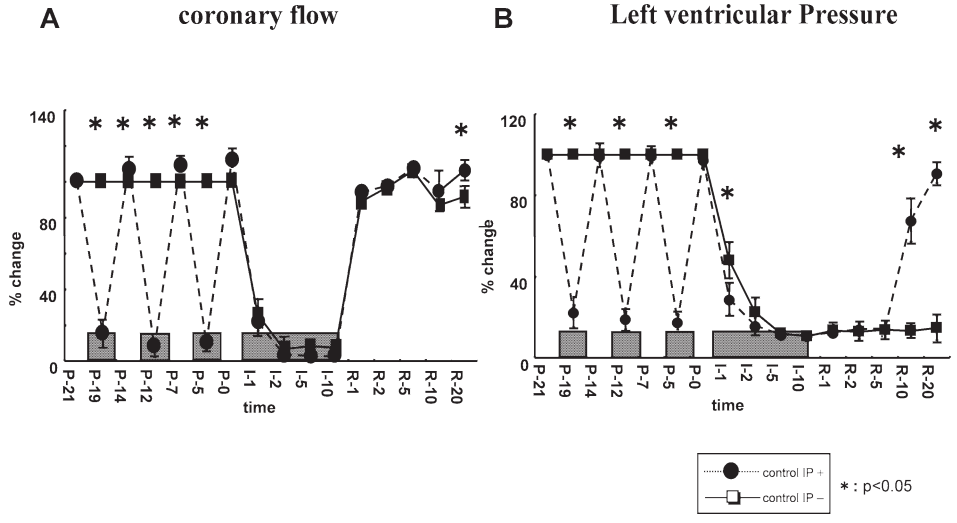

Fig. 5. All left ventricular functional recoveries (A: coronary flow, B: left ventricular pressure) were significantly greater in the control IP+ group than in the control IP− group. Data are shown as means±SEM. *$p<0.05$ compared with IP− group.

IP+ group than in the IP− group (Fig. 5A). In diabetic rats, coronary flow recovery was significantly greater in the IP+ group than in the IP− group at 1, 10, 20 minutes after reperfusion (Fig. 6A).

2) Recovery of left ventricular pressure after reperfusion

In the control rats, left ventricular pressure recovery was significantly greater in the IP+ group than in the IP− group at 1 and 5 minutes after reperfusion (Fig. 6B).

8. Changes in H^+ and lactate levels in coronary effluent during 10-minute ischemia

In control rats, the H^+ level was significantly higher in the IP− group than in the IP+ group after 1, 5, and 10 minutes of ischemia. In diabetic rats, H^+ levels were significantly higher in the IP− group than in the IP+ group after 1, 2, 5, and 10 minutes of ischemia (Fig. 7A, 8A). In control rats, lactate levels were significantly higher in the IP− than in the IP+ group after 1 and 7 minutes of ischemia. In diabetic rats, lactate levels were significantly higher in the IP− group than in the IP+ group after 1 minute of

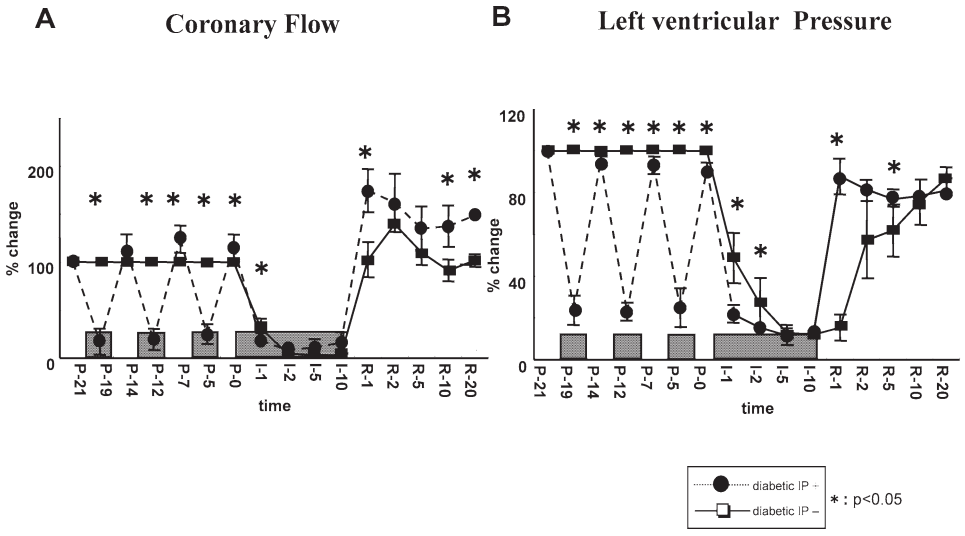

Fig. 6. All left ventricular functional recoveries (A : coronary flow, B : left ventricular pressure) were significantly greater in the diabetic IP+ group than in the diabetic IP− group. Data are shown as means±SEM. *$p<0.05$ compared with IP− group.

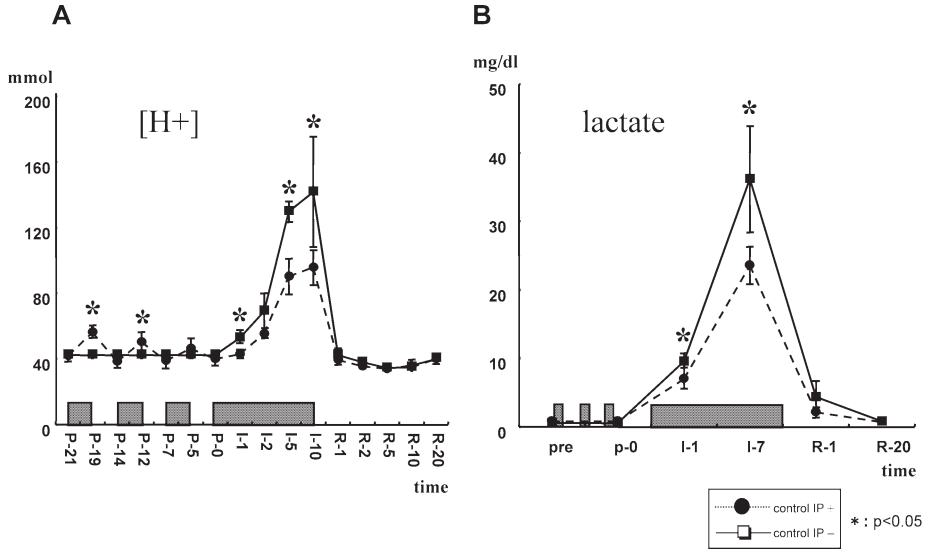

Fig. 7. Time course of changes in H^+ and lactate levels of control IP+ rats and IP− rats 10 minutes of ischemia. H^+ and lactate levels during 10 minutes of ischemia were significantly lower in the control IP+ group than in the control IP− group. Data are shown as means±SEM. *$p<0.05$ compared with IP− group.

ischemia and tended to be higher after 7 minutes of ischemia (Fig. 7A, 8B). The H^+ levels were significantly higher in diabetic rats than in control rats after 5 and 10 minutes of ischemia (Fig. 9).

DISCUSSION

1. Participation of ion transportation system in ischemic reperfusion induced myocardial injury and diabetes mellitus

The ion transportation system may be involved in ischemic reperfusion-induced myocardial injury (Fig. 10). Anaerobic glycolysis generates H^+, which acti-

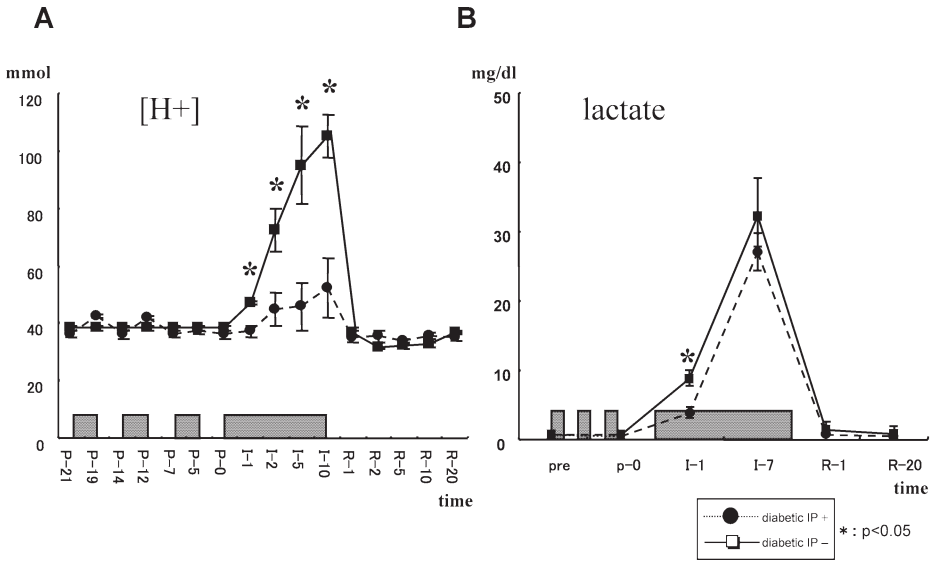

Fig. 8. Time course of changes in H^+ and lactate levels of diabetic IP+ rats and IP− rats during 10 minutes of ischemia. H^+ and lactate levels during 10 minutes of ischemia were significantly lower in the diabetic IP+ group than in the diabetic IP− group. Data are shown as means±SEM. $^*p<0.05$ compared with the IP− group.

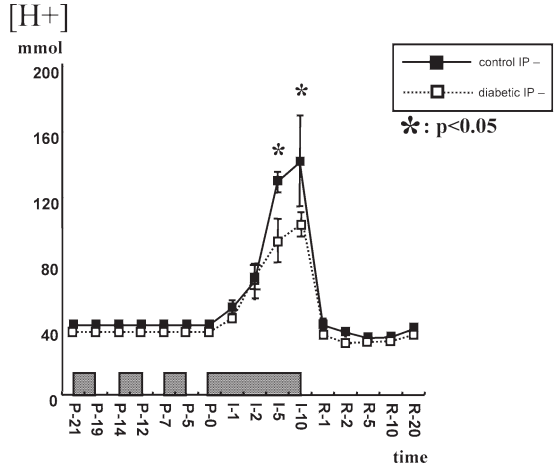

Fig. 9. Time course of changes in H^+ of the diabetic IP− and the control IP− groups during 10 minutes of ischemia. H^+ levels during 10 minutes of ischemia were significantly lower in the diabetic IP− group than in the control IP− group. Data are shown as means±SEM. $^*p<0.05$ compared with IP−.

vates Na+/H^+ exchange (NHE) and Na^+/HCO_3^- co-transport. This, in turn, promotes Na^+ influx, which results in the accumulation of intracellular Na^+ ($[Na^+]_i$). The Na^+/Ca^{2+} exchange (NCX) is pH-sensitive. When the pH decreases because of ischemia, NCX activation is suppressed, which restricts Na^+ discharge against Na^+ accumulation. When acidosis is improved through reperfusion, however, the NCX, which has been suppressed up to this time, again accelerates. This acceleration induces a change in the transmembrane Na^+ gradient, resulting in reverse-mode NCX activity (the opposite of the activity under normal physiological conditions). This reverse-mode activity, in turn, results in extracellular discharge of Na^+. Instead, the NCX causes an influx of Ca^{2+}, producing a $[Ca^{2+}]_i$ overload, which leads to myocardial injury[17].

Suppressed NHE activity has been reported in rats with streptozotocin-induced diabetes[18,19]. The decreased NHE activity suppresses $[Ca^{2+}]_i$ overload, which may prevent ischemia/reperfusion-induced myocardial injury. The suppression of NHE activity may be caused by a decrease in $[Ca^{2+}]_i$ accompanied by functional changes in the cellular membrane[20]. Insulin deficiency accompanies the suppression of NHE activity[21]. Moreover, in experimental diabetic hearts, elevated $[Na^+]_i$ decreases the transmembrane Na^+ gradient, which suppresses NHE activity[22]. Thus, NHE and NCX activity generally decrease in rats with streptozotocin-induced diabetes, which seldom causes $[Ca^{2+}]_i$ overload. This lack of overload

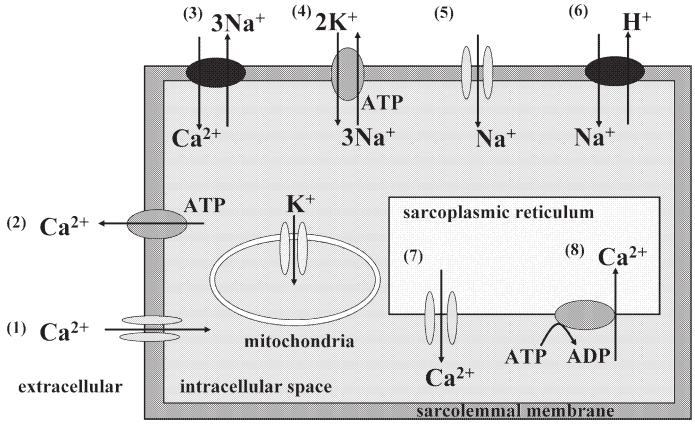

Fig. 10. Cardiac ion transporters that play an important role in ischemia/reperfusion injury.
(1) Ca^{2+} channel; (2) Ca^{2+} pump; (3) NCX; (4) Na^+/K^+ pump; (5) Na^+ channel; (6) NHE; (7) Ca^{2+} release channel; (8) Ca^{2+} pump.

helps reduce ischemia/reperfusion-induced myocardial injury.

2. Attenuated susceptibility to ischemia/reperfusion myocardial injury in rats with streptozotocin-induced diabetes

Our study has shown that the susceptibility to ischemia/reperfusion-induced injury is decreased to a greater extent in rats with streptozotocin-induced diabetes than in control rats. The H^+ level during 10 minutes of ischemia was significantly lower in the IP− group of diabetic rats than in the IP− group of control rats. It has been reported that a major factor in ischemia/reperfusion-induced injury is cellular injury caused by lactate and intracellular acidosis during ischemia[20]. The H^+ changes observed in our study suggest that acidosis may be suppressed during ischemia in the diabetic model rat and that this suppression may increase susceptibility to ischemia/reperfusion-induced myocardial injury. Furthermore, long periods of diabetes mellitus generally decreases NHE and NCX activity[21–24] so that there is little $[Ca^{2+}]_i$ overload. It may be thought that attenuated $[Ca^{2+}]_i$ overload due to decreased activity of NHE and NCX results in a cause of greater tolerance against ischemia.

3. Cardioprotective effect of IP in STZ-induced diabetic rats

This study has confirmed the cardioprotective effects of IP, even in 10-week-old diabetic rats. Tatsumi, et al.[9] have reported that IP promotes recovery of left ventricular function after reperfusion, a finding that confirms the cardioprotective effects of IP in rats with streptozotocin-induced diabetes. The reported mechanism is the suppression of anaerobic glycolysis during long periods of ischemia. Levels of glycogen-degraded endproducts and of H^+ generation during ischemia were lower in diabetic rats than in control rats.

In this study, the generation of H^+ and lactate during a longer period of ischemia (10 minutes) was significantly lower in the IP+ group of diabetic rats than in the IP− group. This finding indicates a suppression of anaerobic glycolysis during a longer period of ischemia, which is similar to the results reported by Tatsumi, et al. The result indicates a suppression of cellular injury caused by lactate and intracellular acidosis during longer periods of ischemia[4], which is a major factor in ischemia/reperfusion-induced myocardial injury.

In other words, our study has shown that IP sufficiently suppresses acidosis and lactate generation even in animal that have had diabetes for longer periods. This suppression may help suppress arrhythmias and recover cardiac function after reperfusion.

4. Role of ATP-sensitive potassium channels (K_{ATP} channels) in the myocardium

Smith, et al.[25] have suggest that the myocardial

K_{ATP} channels in rats with streptozotocin-induced diabetes are more sensitive and open at a higher intracellular ATP level than do the K_{ATP} channels in control rats. Chronic hypoxia in rats with streptozotocin-induced diabetes alters the overall channel activity by modifying the gene expression of the myocardial K_{ATP} channels and affecting the expression levels in the channels. This mechanism may increase tolerance to ischemia/reperfusion-induced arrhythmia[26,27]. However, minute changes in the myocardial K_{ATP} channels in rats with streptozotocin-induced diabetes have not been reported. Future studies should measure the threshold of the channel opening by means of the patch clamp technique and observe the degree of suppression of IP—induced cardioprotection by administering a K_{ATP} channel blocker.

Limitations of this study

In this study, we simultaneously measured the duration of sustained rVT and the recovery of cardiac function after reperfusion as indicators of the cardiprotective effects of IP. In this experimental system, the incidence of rVT was closely related to the recovery of cardiac function; i.e., the presence of rVT greatly contributes to left ventricular function. When rVT appears, cardiac contraction is no longer effective and left ventricular pressure becomes impossible to measure. Simultaneous observation of cardiac function and the duration of sustained arrhythmia is not possible. Therefore, to precisely observe the recovery of cardiac function, we need to either observe rVT during reperfusion after restoring sinus rhythm by means of electric defibrillation or to use another protocol to perform an experiment assuming the absence of arrhythmia.

Rats with streptozotocin-induced diabetes have markedly increased blood glucose, emaciation, and ketoacidosis, which are symptoms similar to those of type 1 diabetes mellitus. On the other hand, ischemic heart diseases develop more often in patients with type 2 diabetes mellitus complicated by obesity, insulin resistance, and hyperlipidemia. This presentation does not correspond to that of the drug-induced diabetic model. Therefore, this study using the model of streptozotocin-induced diabetes does not directly reflect the clinical condition of patients with diabetes.

Conclusion

Our study has shown that IP—induced cardioprotective effects are preserved in older rats that have had streptozotocin-induced diabetes for a longer period. The results also show that these rats are less susceptible to ischemia/reperfusion-induced myocardial injury. Possible mechanisms involved are suppression of the anaerobic glycolysis system and decreased NHE and NCX activity during ischemia, which suppress $[Ca^{2+}]_I$ overload and protect the myocardium.

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