Long-Term Treatment with Glibenclamide Increases Susceptibility of Streptozotocin-Induced Diabetic Rat Heart to Reperfusion-Induced Ventricular Tachycardia

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This study investigated the effects of long-term treatment with glibenclamide (GLIB) on the susceptibility of streptozotocin (STZ)-induced diabetic heart to ischemia/reperfusion insults. Starting 4 weeks after the injection of STZ, rats were treated with GLIB (0.1 mg/kg, ip, three times a week, STZ-GLIB group) or vehicle (STZ-VEH group) for 8 weeks. The recovery of cardiac performance, released creatine kinase (CK), and incidence of ventricular arrhythmias were studied during the reperfusion period in isolated hearts from rats in STZ-GLIB (n = 14) and in STZ-VEH groups (n = 13) and from age-matched control rats (CNT group, n = 14). Each heart was subjected to 5 min of global low-flow ischemia followed by 25 min of no-flow ischemia, with a subsequent 30 min of reperfusion. Plasma glucose level was not significantly different between the STZ-GLIB and STZ-VEH groups. The recovery of cardiac performance and the released CK during reperfusion period were significantly lower in both STZ-VEH and STZ-GLIB groups than in the CNT group ($P < 0.01$ and $P < 0.05$, respectively). Reperfusion resulted in an incidence of ventricular fibrillation in 23% and 21% in STZ-VEH and STZ-GLIB groups, respectively (P = ns). These values were significantly lower than that of the CNT group (100%, $P < 0.001$ for both). More importantly, the incidence of ventricular tachycardia in the STZ-GLIB group was significantly higher than that in the STZ-VEH group (93% vs 54%, $P < 0.05$) and was not significantly different from that in the CNT group (93% vs 100%, P = ns). The results suggest that STZ-induced protection against reperfusion-induced ventricular arrhythmias in diabetic heart may be partially abrogated by long-term treatment with GLIB. Exp Biol Med 228:1234–1238, 2003

Key words: ischemia; reperfusion arrhythmias; streptozotocin; cardioprotection

Diabetes mellitus is associated with cardiovascular complications, resulting in an increased risk of myocardial infarction and congestive heart failure (1). Streptozotocin (STZ)-induced diabetes is widely accepted to be an animal model of insulin-dependent diabetes mellitus (2–6). Several studies have demonstrated that the myocardium of STZ-induced diabetic rats is resistant to ischemia/reperfusion insults (3–5). Compared with normal rats, diabetic rat hearts functionally recover much better from global ischemia (3), are well protected against myocardial infarction (4), and are less susceptible to ischemia/reperfusion ventricular arrhythmias (5). Consistently, we recently reported that STZ-induced diabetic heart shows cardioprotection against ischemia/reperfusion injury and that this protection is associated with augmented resynthesis of high-energy phosphate (HEP) during reperfusion (6).

Glibenclamide (GLIB), a sulfonylurea (SU), is broadly used for a therapeutic approach to controlling blood glucose in patients with Type II diabetes mellitus. The effect of SU on cardiovascular mortality in diabetic patients is controversial (7–10). However, the information available on the effects of long-term treatment with SU on ischemia/reperfusion insults in diabetics is limited. To address this issue, we investigated the effect of long-term treatment with GLIB on the susceptibility to ischemia/reperfusion insults in STZ-induced diabetic rat heart. In the clinical setting, GLIB is not applied to Type I diabetic patients. However, the long-term GLIB treatment in Type II diabetic animals will significantly reduce the blood glucose level, which makes it difficult to compare the response of the hearts to ischemic insults between the GLIB-treated animals and the GLIB-untreated animals, because the plasma glucose levels and the severity of diabetic state are crucial factors for regulating the ischemia/reperfusion injury. The high plasma glucose level will not be altered by the long-term GLIB treatment in STZ-induced diabetic rats, which is naturally expected and rather preferable conditions to assess the effects

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of GLIB because no care is needed for the difference in the hyperglycemic levels and the severity of diabetic state between the two groups. Therefore, the present results using STZ-induced diabetic rats may provide worthwhile information about the effects of long-term GLIB on diabetic heart in response to ischemic insults.

Materials and Methods

Animals. Male Wistar-King rats weighing 180–230 g were made diabetic by a single injection of STZ (60 mg/kg) into the tail vein as previously reported (6). STZ was dissolved in citrate buffer (0.05 *M*, 0.8 ml/kg, pH 4.5). Age-matched control animals received injections of an equivalent volume of citrate buffer as the control (CNT) group. Four weeks after STZ injection, the diabetic rats were defined to be hyperglycemic (>400 mg/dl). At that time, the diabetic rats were divided into the STZ-GLIB and the STZ-vehicle (VEH) groups. The STZ-GLIB group (n = 14) received 0.1 mg/kg of GLIB injected ip (dissolved in 0.1% dimethylsulfoxide). The injection of GLIB was repeated three times a week for 8 weeks. Both the STZ-VEH group (n = 13) and the CNT group (n = 14) were injected with an equivalent volume of 0.1% dimethylsulfoxide for the 8 weeks.

Preparations. The heart was excised between 48–72 hr after the final injection of GLIB or dimethylsulfoxide. All the rats used were heparinized (500 IU/kg, ip) and anesthetized with pentobarbital (50 mg/kg, ip). Before the operation, body weight was measured and blood sample was obtained for the measurement of plasma glucose concentration. The hearts were rapidly removed and placed in ice-cold Krebs-Henseleit buffer until cessation of contraction. The aorta was then cannulated and the coronary vasculature was perfused in the Langendorff mode at a constant pressure of 70 mm Hg. The perfusion medium was Krebs-Henseleit buffer (pH 7.4, in m*M* 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25.0 $NaHCO_3$, 11.0 glucose). The perfusion buffer was bubbled with 95% O_2 and 5% CO_2 at 36°C ± 1°C. Following initiation of Langendorff perfusion, the left atrium was removed and a water-filled latex balloon was cannulated through the mitral orifice into the left ventricle. The heart was allowed to equilibrate for 10 min, following the adjustment of the left ventricular end-diastolic pressure to 5 mm Hg. This balloon volume was maintained throughout the experiment. Electrodes were attached to the right atrium and aorta for artificial pacing at 300 bpm using an electronic stimulator (SEN-7203, Nihon Kohden, Tokyo, Japan). Coronary flow pressure was monitored as hydraulic pressure measured at a level of aortic cannulation. Left ventricular pressure, coronary flow pressure, and electrocardiogram were continuously displayed on a polygraph recorder (WS-681G, Nihon Kohden, Tokyo, Japan) as well as stored on a PCM data recorder (RD-111T, TEAC, Tokyo, Japan) for later analyses. Left ventricular developed pressure (LVDP) was defined as the difference between the left ventricular systolic and diastolic pressure and was used as an index of cardiac performance. All procedures met the guidelines of the Physiological Society of Oita Medical University, Japan, for the care and use of laboratory animals.

Experimental Protocols. During the initial 10 min of constant pressure perfusion, the perfusion flow rate was determined in each heart. This procedure allowed a constant perfusion of each heart at a fixed perfusion rate using a microtube pump (MP-3, Tokyo-Rikakikai, Tokyo). Following a 5-min stabilization period, hearts were paced at 300 bpm using an electronic stimulator (SEN-7203). Low-flow global ischemia was initiated by reducing the perfusion rate to 10% of the baseline flow for 5 min, followed by no flow for 25 min. Pacing was terminated 2 min after the initiation of ischemia. Thereafter, the heart was reperfused for 30 min employing the same perfusion rate used before ischemia. The coronary effluent during the initial 1-min reperfusion was collected to measure creatine kinase (CK) content. Following 30 min of reperfusion, wet ventricular wet weight was measured after removal of the right atrium. A ratio of wet ventricular weight to body weight was calculated. Ventricular arrhythmias during the reperfusion period were defined in accordance with the Lambeth Conventions (11). In brief, ventricular tachycardia (VT) was defined as five or more consecutive premature ventricular complexes. Ventricular fibrillation (VF) was defined as a signal in which individual QRS deflections could no longer be distinguished from one another.

Statistics. All data are described as mean ± SEM. Serial changes of LVDP among three groups were analyzed using two-way analysis of variance (ANOVA) followed by Bonferroni/Dunn test. Incidence of reperfusion-induced ventricular arrhythmias in the three groups was evaluated by χ^2 test. The ratio of CK content to ventricular weight in the three groups was analyzed using one-way ANOVA. A P value < 0.05 was considered significant.

Results

Basic Characteristics of Each Animal Group. Both body weight and wet ventricular weight in the STZ-VEH and the STZ-GLIB groups were lower than those in the CNT group ($P < 0.01$ for each, Table I). The ratio of ventricular wet weight to body weight in the STZ-GLIB group was greater than that in the CNT group ($P < 0.05$), whereas there was no significant difference between the STZ-VEH and CNT groups (Table I). Plasma glucose concentrations in both the STZ-VEH and the STZ-GLIB groups were higher than that in the CNT group ($P < 0.01$ for each, Table I). However, there was no significant difference in this parameter between the STZ-VEH and STZ-GLIB groups.

Coronary Flow Pressure. There was no significant difference either in a ratio of perfusion rate to ventricular wet weight or in coronary flow pressure among the three groups at preischemic period. Neither value of those parameters at 5 min after low-flow ischemia or at 5 min after reperfusion was significantly different (data not shown).

Table I. Body Weight, Ventricular Wet Weight, and Plasma Glucose Concentration in Three Groups at the Time of Study, 12 Weeks after the Injection of Streptozotocin

	CNT (n = 14)	STZ-VEH (n = 13)	STZ-GLIB (n = 14)
Body weight (g)	311 ± 20	206 ± 6[a]	200 ± 6[a]
Ventricular weight (g)	1.28 ± 0.11	0.93 ± 0.04[a]	0.94 ± 0.04[a]
Ventricle/body weight (%)	0.41 ± 0.02	0.45 ± 0.01	0.47 ± 0.02[b]
Plasma glucose (mg/dl)	216 ± 7	674 ± 19[a]	689 ± 25[a]

Note. Values are mean ± SEM. CNT, control; STZ, streptozotocin; VEH, vehicle; GLIB, glibenclamide. See text for classification of the three groups.
[a] $P < 0.01$ vs control group.
[b] $P < 0.05$.

Left Ventricular Function. At baseline recording with pacing, LVDP in both STZ-VEH and STZ-GLIB groups was significantly lower than that in the CNT group ($P < 0.05$ for each, Fig. 1), whereas no significant difference was observed between STZ-VEH and STZ-GLIB groups. During low-flow ischemia, the decrease in LVDP was similar among the three groups. During reperfusion, the recovery of LVDP was significantly greater in the STZ-VEH and STZ-GLIB groups compared with the CNT group ($P < 0.05$ and $P < 0.01$, respectively). However, there was no significant difference between the STZ-VEH and STZ-GLIB groups.

Released CK. Figure 2 illustrates the ratio of CK content in coronary effluent obtained during the initial 1 min of reperfusion period to ventricular weight in the three groups. The values of both STZ-VEH and STZ-GLIB groups were significantly lower than that of CNT group ($P < 0.05$ for each). However, no significant difference was observed between the STZ-VEH and STZ-GLIB groups.

Incidence of Reperfusion-Induced Ventricular Arrhythmias. Figure 3 shows incidence of reperfusion-induced ventricular arrhythmias in each group. Both VF and VT were developed at 100% in the CNT group. Incidences of VF in the STZ-VEH (23%) and the STZ-GLIB (21%) groups were lower than that in the CNT group ($P < 0.01$ for each). Incidence of VT in the STZ-VEH group was lowered by 54% and this reduction was significant compared with the CNT group ($P < 0.01$). In contrast, incidence of VT in the STZ-GLIB group (93%) did not differ from that in the CNT group (100%) and was greater than that in the STZ-VEH group ($P < 0.05$).

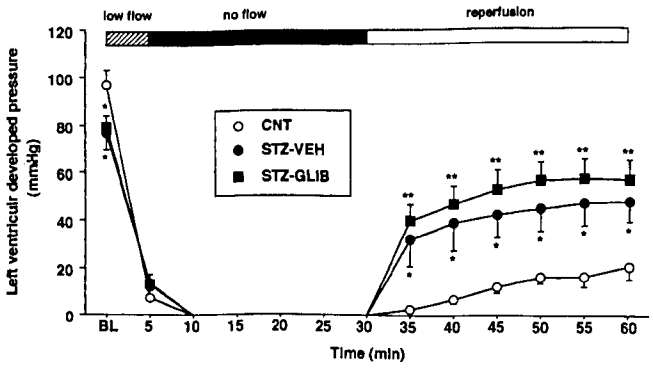

Figure 1. LVDP during low-flow, no-flow, and reperfusion periods. CNT, control; STZ, streptozotocin; VEH, vehicle; GLIB, glibenclamide. *$P < 0.05$, **$P < 0.01$.

Discussion

Main Findings. The present study showed that STZ-induced diabetic rat hearts were protective against ischemia/reperfusion injury when assessed by left ventricular functional recovery, released CK, and incidence of ventricular arrhythmias. The long-term treatment with GLIB in STZ-induced diabetic heart produced no significant changes in functional recovery and released CK. With respect to the susceptibility to reperfusion-induced ventricular arrhythmias, the long-term GLIB in STZ-induced diabetic heart did not influence the incidence of reperfusion-induced VF but increased the incidence of reperfusion-induced VT to the comparable level with age-matched control group. Pancreatic β-cells in STZ-induced diabetes were destroyed and could not therefore secrete insulin in response to SU agents.

In fact, blood glucose concentrations in the diabetic rats were not affected in the present study by long-term treat-

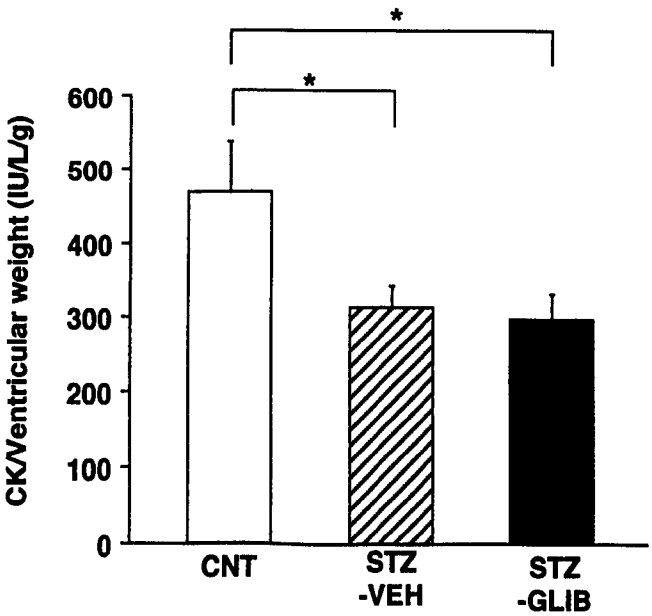

Figure 2. CK released during no-flow ischemia. The CK content was measured in the coronary effluent obtained during the initial 1 min of reperfusion. Values were expressed as the ratio of CK content to wet ventricular weight. CNT, control; STZ, streptozotocin; VEH, vehicle; GLIB, glibenclamide. *$P < 0.05$.

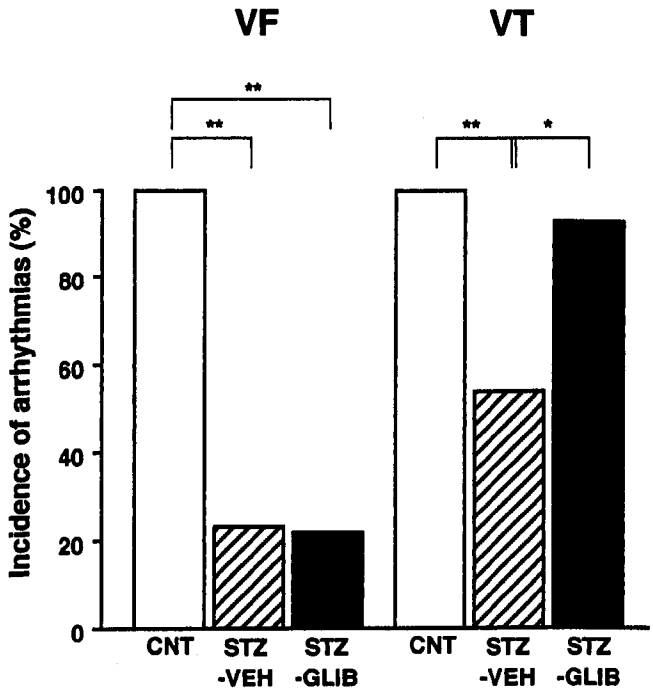

Figure 3. Incidence of reperfusion-induced ventricular arrhythmias. CNT, control; STZ, streptozotocin; VEH, vehicle; GLIB, glibenclamide; VF, ventricular fibrillation; VT, ventricular tachycardia. $^{*}P < 0.05$, $^{**}P < 0.01$.

ment with GLIB. In addition, the hearts were excised 48–72 hr after the final administration of GLIB, which was approximately 10 times a half-life of GLIB. Taken together, the differences in the present results between the STZ-GLIB and the STZ-VEH groups depended on the chronic effects of GLIB.

Mechanisms for Cardioprotection in Diabetic Hearts. It remains unclear what mechanisms are involved in the effects of STZ-induced diabetic state on cardioprotection. We recently reported that STZ-induced diabetic heart shows cardioprotection against ischemia/reperfusion injury, when assessed by functional recovery and released CK, and that this protection may be associated with augmented resynthesis of HEP during reperfusion (6). On the other side, altered intracellular calcium ($[Ca^{2+}]_i$) metabolism may contribute to the STZ-induced diabetic cardioprotection. Reduced activities of the Na^+/H^+ pump and Na^+/Ca^{2+} exchanger were demonstrated in STZ-induced diabetic hearts (12–14), indicating less accumulation of Na^+ and Ca^{2+} in the myocardium during ischemia and reperfusion. Indeed, lowered activities of the Na^+/H^+ pump and Na^+/Ca^{2+} exchanger were found to promote cardioprotection against an ischemic insult in STZ-induced diabetic hearts (15). Inhibition of $[Ca^{2+}]_i$ overload may lessen myocardial damage with ischemia/reperfusion and increase net HEP resynthesis at reperfusion.

The susceptibility of STZ-induced diabetic rat heart to reperfusion-induced ventricular arrhythmias has been reported previously (5, 16). Diabetic rat heart was resistant to reperfusion-induced ventricular arrhythmias, which was studied 3 weeks after STZ injection (5). Another group (16) also observed less susceptibility to reperfusion-induced ventricular arrhythmias in the early phase of diabetes (2 weeks after STZ injection). The present study performed at 12 weeks after STZ injection, therefore, confirms that cardioprotection is evident even after a relatively long period of induction of diabetes.

Effects of Long-Term Treatment with Glibenclamide. It was previously reported that, in isolated perfused STZ-induced diabetic heart, the incidence of reperfusion-induced arrhythmias was reduced by GLIB applied into perfusate for 10 min before the onset of ischemia and during reperfusion (16). The long-term administration of GLIB into diabetic rats in the present study, in contrast, resulted in an increased incidence of reperfusion-induced VT. However, the incidence of VF was not different between the STZ-VEH and STZ-GLIB groups. These findings suggest the difference in underlying electrophysiological mechanisms between VT and VF observed during reperfusion. VF was probably caused by the reentrant mechanism. In contrast, $[Ca^{2+}]_i$ overload brought by ischemia and subsequent reperfusion prefers to produce arrhythmias triggering from delayed afterdepolarization, which may be manifested as VT rather than VF. Therefore, it can be speculated that long-term treatment with GLIB enhances $[Ca^{2+}]_i$ overload during the reperfusion period. In the present study, the final administration of GLIB was 48–72 hr before heart excision, at which time the acute effect of GLIB to block K_{ATP} channel was considered to disappear. It remains to be studied whether the ischemia-induced activation of K_{ATP} channel in diabetic heart could be modulated by long-term treatment with GLIB. Besides blocking K_{ATP} channel, the SU agents possess other effects that could affect the cardiovascular systems. For example, the SU agents inhibit the function of CFTR (cystic fibrosis transmembrane conductance regulator), altering the effects of ischemia on intracellular Cl^-, Na^+, and pH (17). They also increase tissue plasminogen activator production by endothelial cells, inhibit platelet function, and effect net K^+ balance in the kidney (18, 19). In addition, the SU agents might activate cleavage of 5′-nucleotidase by activation of a phosphatidyl inositol/phospholipase C, thus lowering effective adenosine concentrations near the adenosine receptor (20). The combination of these complex effects of GLIB may increase the susceptibility of diabetic hearts to reperfusion-induced ventricular arrhythmias.

Implications. In the early 1970s, the University Group Diabetes Problem (UGDP) assessed the efficacy of SU treatment in comparison with insulin and diet alone in the prevention of vascular complications and revealed a significantly higher cardiovascular mortality in patients on SU compared with those on diet alone (7). The present study for the first time demonstrated an increase in incidence of reperfusion-induced VT in diabetic rats chronically treated with GLIB. The observation may shed light on the adverse effects of long-term treatment with SU drugs in patients

with non–insulin dependent diabetes mellitus as observed in the UGDP study. However, recently published United Kingdom Prospective Diabetes Study (UKPDS) results found no increase in cardiovascular mortality with the use of SU (10). The difference between the results of the UGDP and UKPDS may be due to the exclusion of patients with cardiovascular diseases in the UKPDS. Future study will be needed to assess the long-term administration of SU on cardiac mortality in patients with cardiovascular diseases.

Limitations. In the present study, we investigated the effects of long-term treatment with GLIB in STZ-induced diabetic rat heart. In our series of experiments, STZ-induced diabetic heart showed protection against ischemic insults. It has been also reported that the heart of non–insulin dependent diabetic rats was protected from infarction (4). However, there have been no clinical reports that demonstrated the existence of cardioprotection in Type II diabetic patients. It remains to be studied whether the adverse effects of long-term treatment with GLIB as demonstrated in the present study would be observed in the Type II diabetic animals and patients.

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