Original Article

Pioglitazone but Not Glibenclamide Improves Cardiac Expression of Heat Shock Protein 72 and Tolerance Against Ischemia/Reperfusion Injury in the Heredity Insulin-Resistant Rat

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We tested the hypothesis that pioglitazone could restore expression of heat shock protein (HSP)72 in insulin-resistant rat heart. At 12 weeks of age, male Otsuka Long-Evans Tokushima Fatty (OLETF) rats and control (LETO) rats were treated with pioglitazone (10 mg · kg^{-1} · day^{-1}) or glibenclamide (5 mg · kg^{-1} · day^{-1}) for 4 weeks. Thereafter, hyperthermia (43°C for 20 min) was applied. In response to hyperthermia, the activation of serine/threonine kinase Akt depending on phosphatidylinositol 3 (PI3) kinase was necessary for cardiac expression of HSP72. Hyperthermia-induced activation of Akt and HSP72 expression were depressed in OLETF rat hearts. Pioglitazone but not glibenclamide improved insulin sensitivity in OLETF rats, which was associated with the restoration of Akt activation and HSP72 expression. In experiments with isolated perfused heart, reperfusion-induced cardiac functional recovery was suppressed in OLETF rat hearts, which was improved by pioglitazone but not glibenclamide. Our results suggest that PI3 kinase–dependent Akt activation, an essential signal for HSP72 expression, is depressed in the heart in insulin-resistant OLETF rats, and the results suggest also that the restoration of HSP72 expression and tolerance against ischemia/reperfusion injury by treatment with pioglitazone might be due to an improvement of insulin resistance, leading to restoration of impaired PI3 kinase–dependent Akt activation in response to hyperthermia. *Diabetes* 55:2371–2378, 2006

Heat shock protein (HSP)72, a member of the HSP family, has been reported to be involved predominantly in cardioprotection (1–4). We have shown that induction of HSP72 by either hyperthermia or oral administration of geranylgeranylacetone (GGA) leads to protection against ischemia/reperfusion injury (5–8). However, information is very limited with respect to the impact of insulin resistance and type 2 diabetes on the expression of HSP72. Kurucz et al. (9) reported that the expression of HSP72 mRNA in skeletal muscle of patients with type 2 diabetes is decreased, which correlates with the parameters of insulin resistance. Consistent with this, we have shown attenuated cardiac HSP72 expression in response to GGA in insulin-resistant rats produced by a high-fat diet (10). Skeletal muscle, liver, and heart are known to be targeted organs of insulin resistance (11–13). However, the mechanisms underlying the depressed expression of HSP72 in insulin-resistant organs are unclear. Recently, thiazolidinediones, including pioglitazone, have been demonstrated to improve insulin resistance experimentally and clinically (14,15). Whereas sulfonylurea including glibenclamide stimulates endogenous insulin secretion through blockade of ATP-sensitive potassium channels on pancreatic β-cells, pioglitazone, a synthetic peroxisome proliferator–activated receptor-γ (PPAR-γ) agonist, does not stimulate insulin release from pancreatic β-cells (14,15). It is therefore interesting to compare the effects of treatment with pioglitazone and treatment with glibenclamide on the expression of HSP72 in the insulin-resistant animal model.

Activation of the serine/threonine kinase Akt promotes survival of some cell types including cardiomyocytes (16). Hyperthermia is known to cause Akt activation, and experimental evidence indicates that hyperthermia-induced activation of Akt can involve either a phosphatidylinositol 3 (PI3) kinase–dependent or a PI3 kinase–independent pathway (17,18). However, little is known about the involvement of Akt in hyperthermia-induced HSP72 expression in the heart. We have demonstrated recently that PI3 kinase–dependent Akt activation is essential for expression of HSP72 (19). In the present study, therefore, we tested the hypothesis that *1*) depressed cardiac HSP72 expression in insulin-resistant rats is related to depressed PI3 kinase–dependent Akt activation in response to hyperthermia and that *2*) treatment with pioglitazone improves this depressed response, resulting in restoration of cardiac expression of HSP72, which is associated with the protection against ischemia/reperfusion injury.

RESEARCH DESIGN AND METHODS

All experimental procedures were carried out in accordance with the guidelines of the Physiological Society of Oita University, Japan, for the care and use of laboratory animals.

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CPP, coronary perfusion pressure; GGA, geranylgeranylacetone; HSP, heat shock protein; LVDP, left ventricular developed pressure; OGTT, oral glucose tolerance test; PI3, phosphatidylinositol 3; PPAR-γ, peroxisome proliferator–activated receptor-γ; STZ, streptozotocin.

Received for publication 25 February 2006 and accepted in revised form 22 May 2006.

DOI: 10.2337/db06-0268

TABLE 1
Basic characteristics of experimental groups after 4-week treatment with vehicle (VEH), pioglitazone (PIO), or glibenclamide (GLIB)

	LETO-VEH	LETO-PIO	LETO-GLIB	OLETF-VEH	OLETF-PIO	OLETF-GLIB	*P* value
n	14	12	12	14	12	12	
Body weight (g)	339.8 ± 4.6	346.4 ± 6.0	357.6 ± 6.9	383.4 ± 5.3*	377.6 ± 7.7*	423.5 ± 4.7*§	<0.001
LV weight (g)	0.91 ± 0.02	0.88 ± 0.03	0.96 ± 0.05	1.03 ± 0.02*	1.16 ± 0.31*	1.15 ± 0.03*§	<0.001
LV/body weight (mg/g)	2.58 ± 0.06	2.52 ± 0.09	2.73 ± 0.19	2.59 ± 0.05	2.81 ± 0.15	2.73 ± 0.06	NS
Plasma glucose (mmol/l)	5.02 ± 0.16	5.08 ± 0.29	5.08 ± 0.29	5.14 ± 0.38	4.44 ± 0.21	4.93 ± 0.25	NS
Plasma insulin (ng/ml)	1.94 ± 0.53	1.72 ± 0.50	3.32 ± 0.60	5.24 ± 1.19*	2.69 ± 0.51‡	4.79 ± 0.87	<0.01
Total cholesterol (mg/dl)	75.2 ± 1.6	73.9 ± 4.5	71.8 ± 3.0	73.5 ± 3.0	72.1 ± 5.1	72.0 ± 6.2	NS
Triglycerides (mg/dl)	32.9 ± 3.8	30.2 ± 2.2	36.0 ± 4.5	61.1 ± 6.2*	54.2 ± 2.5*	62.9 ± 6.1*	<0.001
Free fatty acid (mg/dl)	397.4 ± 21.9	348.2 ± 34.0	381.1 ± 31.0	639.5 ± 73.9†	606.4 ± 61.2*	724.0 ± 53.0*	<0.001

Data are means ± SE. *$P < 0.01$, †$P < 0.05$ vs. corresponding LETO groups; ‡$P < 0.05$, §$P < 0.01$ vs. corresponding vehicle-treated groups. LV, left ventricle.

Antibody to HSP72 (mouse) was purchased from Stressgen Biotechnologies. Antibodies to Akt, phospho-Ser^{473}-Akt, and phospho-Thr^{311}-Akt were purchased from Cell Signaling Technology. Horseradish peroxidase–tagged secondary antibodies and enhanced chemiluminescence reagents were purchased from Amersham Pharmacia Biotech. Bradford protein assay kits were purchased from Bio-Rad. Wortmannin and other chemical agents were purchased from Sigma Chemical.

Four-week-old male Otsuka Long-Evans Tokushima Fatty (OLETF) rats (70–80 g initial weight) were provided by the animal center of Tokushima Research Institute (Otsuka Pharmaceuticals). Age-matched Long-Evans Tokushima Otsuka (LETO) rats, which were developed from the same colony but do not develop insulin resistance, were used as normal controls. All rats were housed in a room illuminated daily from 700 to 1900 (12:12-h light/dark cycle) with temperature maintained at 21 ± 1°C. They were allowed free access to tap water and standard pellet rat diet. At 12 weeks of age, OLETF ($n = 90$) and LETO ($n = 90$) rats were introduced to be treated with pioglitazone (10 mg · kg^{-1} · day^{-1}, OLETF-PIO or LETO-PIO group, $n = 30$ for each), glibenclamide (5 mg · kg^{-1} · day^{-1}, OLETF-GLIB or LETO-GLIB group, $n = 30$ for each), or vehicle (OLETF-VEH or LETO-VEH group, $n = 30$ for each) orally for 4 weeks. At 16 weeks of age, each rat was anesthetized with pentobarbital (20 mg/kg i.p.) and immersed in a water bath at 43°C (hyperthermia) or 37°C (normothermia, NT) for 20 min. Rectal temperature was monitored throughout the thermo treatment to confirm changes in body temperature. In addition, some rats of the hyperthermia group ($n = 4$ for each group) were injected with either wortmannin (16 μg/kg i.v.) or saline ($n = 4$ for each group) into a tail vein 15 min before hyperthermia. Twenty-four hours after thermo treatment with normothermia or hyperthermia, following anesthesia with pentobarbital (20 mg/kg i.p.), rat hearts were isolated and prepared for Western blot analysis ($n = 4$ for each group) or isolated perfused heart experiments ($n = 7$ for each group).

Oral glucose tolerance test. At 16 weeks of age, an oral glucose tolerance test (OGTT) was performed after an overnight fast ($n = 8$ for each group). Glucose solution (2 g/kg) was administered orally, and at 0, 30, 60, 90, and 120 min, blood was drawn from a tail vein. The plasma glucose concentration was measured with a commercial test kit (GR-102; Terumo). The plasma insulin concentration was quantified using an enzyme-linked immunosorbent assay insulin kit (Morinaga Seikagaku).

Western blot analysis. Western blot analysis was performed as described (5,7,19). Briefly, rats were heparinized (500 IU/kg i.p.) and anesthetized with pentobarbital (50 mg/kg i.p.). Each heart was removed rapidly and frozen in liquid nitrogen. The frozen tissues were homogenized with lysis buffer (50 mmol/l Tris-HCl [pH 7.4], 10% glycerol, 2 mmol/l EDTA, 150 mmol/l NaCl, 1 mmol/l $MgCl_2$, 50 mmol/l glycerophosphate, 2 mmol/l Na_3VO_4, 20 mmol/l NaF, 1 mmol/l phenylmethylsulfonylfluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 1% Nonidet P-40). Samples were centrifuged and the concentration of protein was measured by the Bradford method (20). An equal amount of total protein in each fraction was subjected to SDS-PAGE and transferred electrophoretically onto a polyvinylidine fluoride membrane. After blocking with 0.5% nonfat milk, the membranes were incubated with antibodies. After repeated washing, the membranes were incubated with secondary antibodies. The proteins were detected by enhanced chemiluminescence following exposure to Hyperfilm. The amount of protein on the immunoblots was quantified using National Institutes of Health image analysis software.

Isolated perfused heart experiments. Isolated perfused experiments using a Langendorff apparatus were performed as described (5,7,19). At 24 h after hyperthermia ($n = 7$ for each group) or normothermia ($n = 7$ for each group), the heart was isolated and perfused retrogradely with Krebs-Henseleit buffer (pH 7.4; 118 mmol/l NaCl, 4.7 mmol/l KCl, 2.5 mmol/l $CaCl_2$, 1.2 mmol/l $MgSO_4$, 1.2 mmol/l KH_2PO_4, 25.0 mmol/l Na_2HCO_3, 11.0 mmol/l glucose, 100 μU/ml insulin, and 3% BSA) equilibrated with 95% O_2/5% CO_2 gas mixture at 36.5°C at a constant pressure of 75 mmHg. A water-filled latex balloon was inserted through the mitral valve orifice into the left ventricle, and the left ventricular end-diastolic pressure was adjusted to 0 mmHg. During the initial 10 min of constant pressure perfusion, the perfusion flow rate was determined for each heart, which was then perfused at a determined perfusion rate using a microtube pump, while the heart was covered with water-jacketed glassware and the relative humidity was maintained at 90% or more. No-flow global ischemia was introduced for 20 min, followed by reperfusion for 30 min. The coronary effluent during the 30-min reperfusion period was collected for measurement of creatine kinase content. Creatine kinase activity was determined with a spectrophotometer by measuring NADPH production as the change in absorbance at 340 nm in the solution containing 2 mmol/l ADP, 20 mmol/l glucose, and 2 mmol/l NADP, along with 2 IU/ml of both hexokinase and glucose-6-phosphate dehydrogenase at a pH of 7.1. The reaction was initiated by addition of creatine phosphate (21). The ratio of released creatine kinase to left ventricular weight was calculated. Left ventricular pressure was monitored using a pressure transducer to obtain the peak positive and negative first derivatives of left ventricular pressure (dP/dt_{max} and dP/dt_{min}). Left ventricular developed pressure (LVDP) was defined as the difference between the left ventricular systolic and diastolic pressure. Left ventricular pressure, coronary perfusion pressure (CPP), and electrocardiogram were recorded continuously on a polygraph recorder (WS-681G; Nihon Kohden) and stored on a PCM data recorder (RD-111T; TEAC) for later analysis.

Statistical analysis. Data are expressed as means ± SE. Serial changes in plasma concentrations of glucose and insulin during OGTT and LVDP, left ventricular end-diastolic pressure, dP/dt, CPP, and heart rate during the reperfusion period were analyzed by two-way ANOVA followed by the Bonferroni-Dunn test. Comparison of physiological and serum parameters, the relative intensity of each protein, and the ratio of released creatine kinase to left ventricular weight was analyzed using one-way ANOVA, followed by the Bonferroni-Dunn test. A P value <0.05 was considered statistically significant.

RESULTS

Physical and metabolic characteristics. Table 1 summarizes the physical and metabolic parameters after overnight fasting in the LETO and OLETF rats after 4 weeks of treatment with vehicle, pioglitazone, or glibenclamide. When compared with the LETO-VEH group, body weight and left ventricular weight were greater in the OLETF-VEH group ($P < 0.01$ for each). Although the plasma glucose concentrations were not significantly different, the plasma insulin concentrations were higher in the OLETF-VEH group than in the LETO-VEH group ($P < 0.01$). With regard to the indexes of lipid metabolism, although the total cholesterol concentration was not significantly different, concentrations of plasma triglycerides and free fatty acid were higher in the OLETF-VEH group than in the LETO-VEH group ($P < 0.01$ and $P < 0.05$, respectively). Four weeks of treatment with pioglitazone or glibenclamide did not influence any physical and metabolic parameter in the LETO rats. In the OLETF rats, when compared with the OLETF-VEH group, the OLETF-PIO group had lower

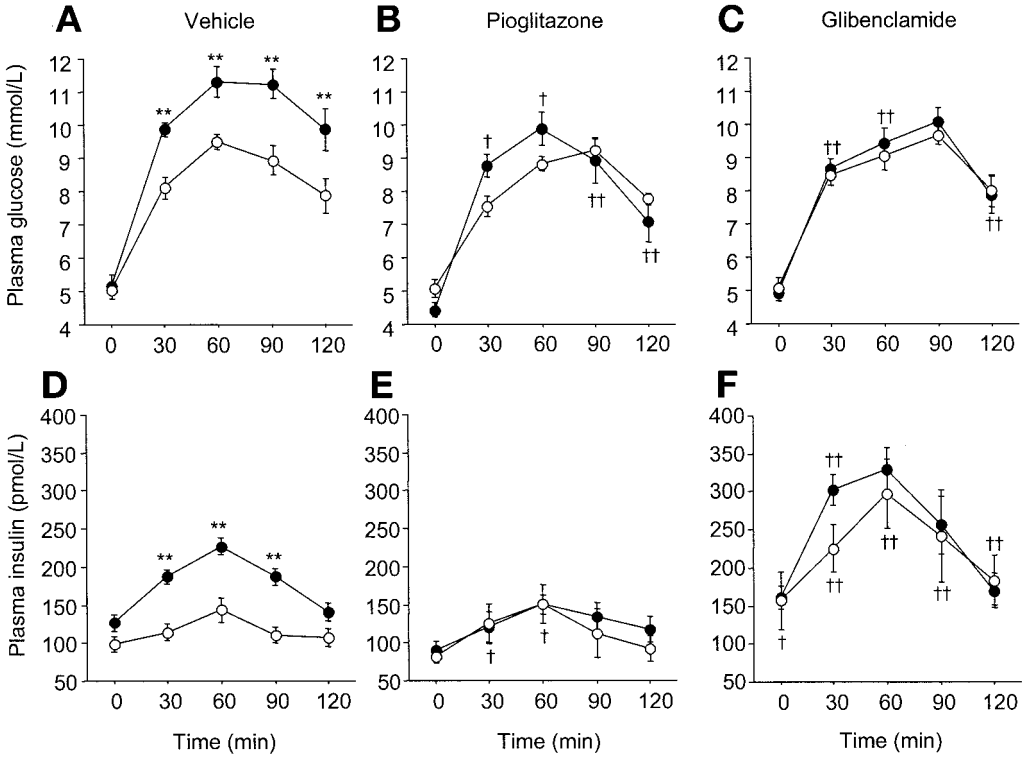

FIG. 1. Time courses in blood glucose (*A–C*) and plasma insulin (*D–F*) concentrations during OGTT. Data are mean ± SE. *A* and *D*: ○, LETO-VEH; ●, OLETF-VEH. *B* and *E*: ○, LETO-PIO; ●, OLETF-PIO. *C* and *F*: ○, LETO-GLIB; ●, OLETF-GLIB. ***P* < 0.01 vs. corresponding LETO groups. †*P* < 0.05, ††*P* < 0.01 vs. corresponding vehicle-treated groups.

plasma insulin concentrations, while no significant difference was observed in plasma glucose concentrations or indexes of lipid metabolism. In contrast, when compared with the OLETF-VEH group, the OLETF-GLIB group had higher body weight and left ventricular weight while no significant difference was observed in plasma glucose, insulin concentrations, or indexes of lipid metabolism.

OGTT. Figure 1 plots the plasma glucose and insulin concentrations during the OGTT. The increase of plasma glucose and insulin in response to oral glucose was more pronounced in the OLETF-VEH group than in the LETO-VEH group ($P < 0.01$ for each). Treatment with pioglitazone suppressed the increase of plasma glucose and insulin in the OLETF rats ($P < 0.01$ and $P < 0.05$ for each, respectively), resulting in no significant difference between the OLETF-PIO group and the LETO-PIO group. Treatment with glibenclamide also suppressed the increase of plasma glucose in the OLETF rats ($P < 0.01$). However, the treatment rather enhanced the increase of plasma insulin ($P < 0.05$).

Cardiac HSP72 expression and PI3 kinase dependence. Figure 2 depicts the cardiac HSP72 expression at 24 h after each thermo treatment in vehicle-treated rats. Hyperthermia induced HSP72 expression in both the LETO-VEH and the OLETF-VEH groups ($P < 0.01$ and $P < 0.05$, respectively). However, the levels of expression in OLETF-VEH group was less when compared with that in the LETO-VEH group ($P < 0.01$). The pretreatment with wortmannin inhibited the expression of HSP72 specifically in the LETO-VEH group ($P < 0.01$), resulting in no significant difference between the LETO-VEH group and the OLETF-VEH group.

Cardiac Akt phosphorylation and PI3 kinase dependence. Figure 3 depicts the phosphorylation of Akt at 1 h after each thermo treatment in vehicle-treated rats. The three representative bands for total Akt, phospho-Ser^{473}-

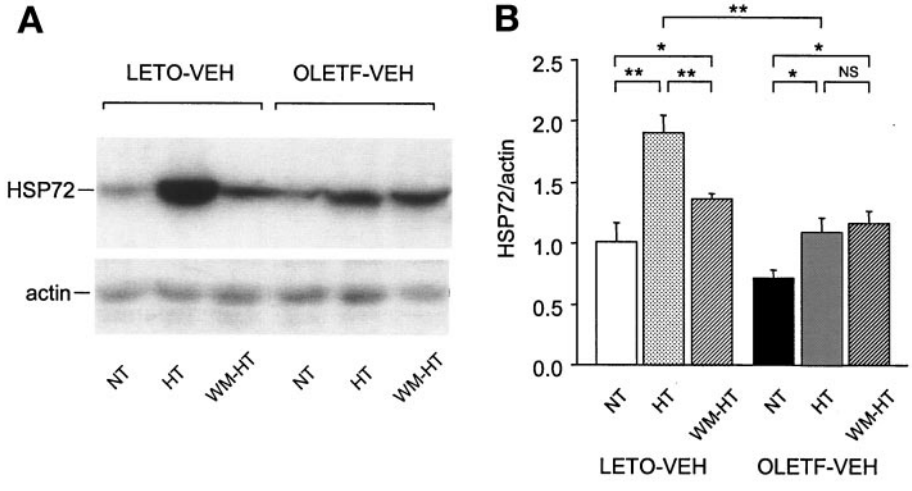

FIG. 2. Cardiac expression of HSP72 in LETO-VEH and OLETF-VEH groups. The heart was isolated 24 h after each thermo treatment. Representative immunoblot bands (*A*) and quantification of the ratio of HSP72 to actin (*B*). *n* = 4 for each group. Data are means ± SE. HT, hyperthermia; NT, normothermia; WM-HT, wortmannin pretreatment followed by HT. **P* < 0.05, ***P* < 0.01.

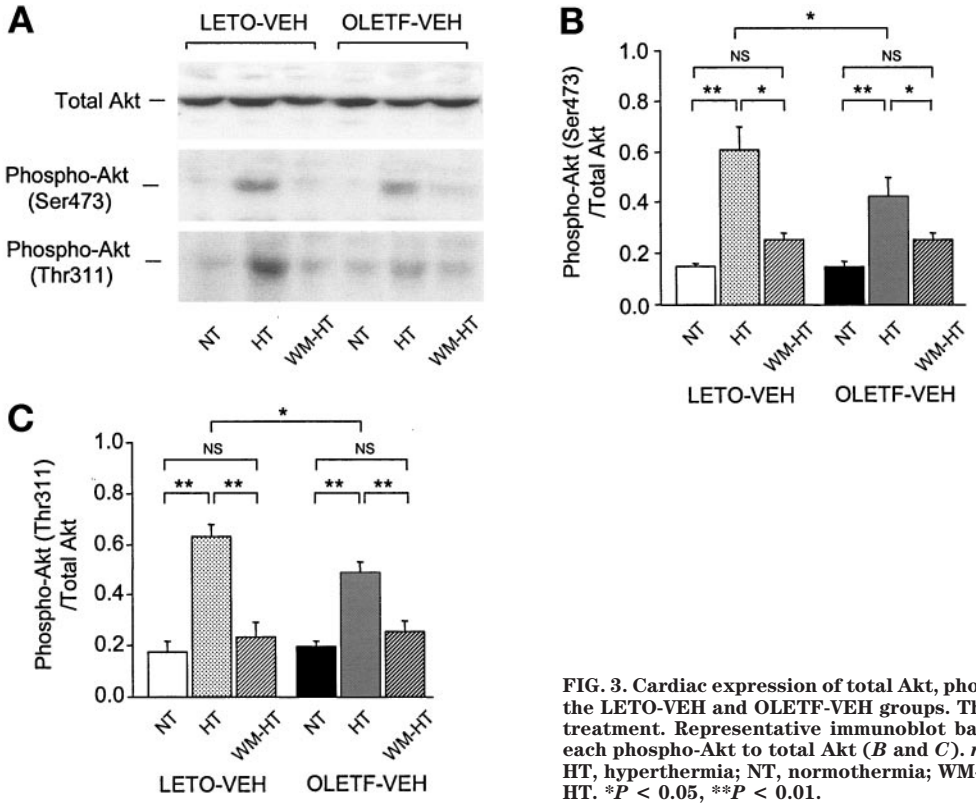

FIG. 3. Cardiac expression of total Akt, phospho-Ser^{473}-Akt, and phospho-Thr^{311}-Akt in the LETO-VEH and OLETF-VEH groups. The heart was isolated 1 h after each thermo treatment. Representative immunoblot bands (*A*) and quantification of the ratio of each phospho-Akt to total Akt (*B* and *C*). $n = 4$ for each group. Data are means ± SE. HT, hyperthermia; NT, normothermia; WM-HT, wortmannin pretreatment followed by HT. *$P < 0.05$, **$P < 0.01$.

Akt, and phospho-Thr^{311}-Akt are shown. Hyperthermia induced Akt phosphorylation in both the LETO-VEH and the OLETF-VEH groups ($P < 0.01$ for each). However, phosphorylation in the OLETF-VEH group was less when compared with that in the LETO-VEH group ($P < 0.05$). The pretreatment with wortmannin inhibited the Akt phosphorylation in both groups ($P < 0.05$ for each).

Reperfusion-induced left ventricular functional recovery. Figure 4*A* and *B* illustrate the serial changes in LVDP during the experimental period in the LETO-VEH and the OLETF-VEH groups. LVDP, heart rate, and CPP did not show a significant difference among the four experimental groups during the baseline period (data not shown). During no-flow global ischemia, LVDP decreased rapidly to zero. Hyperthermia, when compared with normothermia, improved left ventricular functional recovery in the LETO-VEH group ($P < 0.05$). However, the improvement did not reach statistical significance in the OLETF-VEH group (Fig. 4*A*). The improvement induced by hyperthermia in the LETO-VEH group was almost suppressed by the pretreatment with wortmannin (Fig. 4*B*). Figure 4*C* shows the amount of creatine kinase released during the 30 min of reperfusion period, which was greater in the OLETF-VEH group than in the LETO-VEH group ($P < 0.05$). Hyperthermia reduced the amount of released creatine kinase in the LETO-VEH group ($P < 0.05$), but the reduction did not reach statistical significance in the OLETF-VEH group. The pretreatment with wortmannin diminished the hyperthermia-induced reduction of released creatine kinase observed for the LETO-VEH group.

Effects of treatment with pioglitazone or glibenclamide on cardiac HSP72 expression. Figure 5 shows the effects of treatment with pioglitazone or glibenclamide on the expression of cardiac HSP72. Treatment with pioglitazone or glibenclamide did not influence the expression of HSP72 in the LETO groups. The observed depressed expression of HSP72 induced by hyperthermia in the OLETF-VEH group compared that in the LETO-VEH group ($P < 0.01$) was restored by treatment with pioglitazone. In contrast, treatment with glibenclamide resulted in failure to restore HSP72 expression.

Effects of pioglitazone treatment on Akt phosphorylation. Figure 6 depicts the effects of treatment with pioglitazone on Akt phosphorylation at 1 h after hyperthermia application. Treatment with pioglitazone restored Akt phosphorylation in the OLETF-VEH group, resulting in no significant difference between the LETO-PIO group and the OLETF-PIO group. The hyperthermia-induced Akt phosphorylation was suppressed by the pretreatment with wortmannin.

Effects of treatment with pioglitazone or glibenclamide on reperfusion-induced left ventricular functional recovery. Figure 7*A* and *B* illustrate the serial changes in LVDP during the experimental period. LVDP, heart rate, and CPP did not show a significant difference among the four experimental groups during the baseline period (data not shown). During no-flow global ischemia, LVDP decreased rapidly to zero. Hyperthermia improved left ventricular functional recovery in the LETO-PIO group and the OLETF-PIO group ($P < 0.01$ for each), although the improvement was more pronounced in the LETO-PIO group than that in the OLETF-PIO group ($P < 0.05$, Fig. 7*A*). In contrast, hyperthermia improved left ventricular functional recovery in the LETO-GLIB group ($P < 0.05$) but not in the OLETF-GLIB group (Fig. 7*B*). As shown in Fig. 7*C*, hyperthermia resulted in comparable levels of released creatine kinase between the LETO-PIO and the OLETF-PIO groups. However, hyperthermia did not reduce the amount of released creatine kinase in the OLETF-GLIB group.

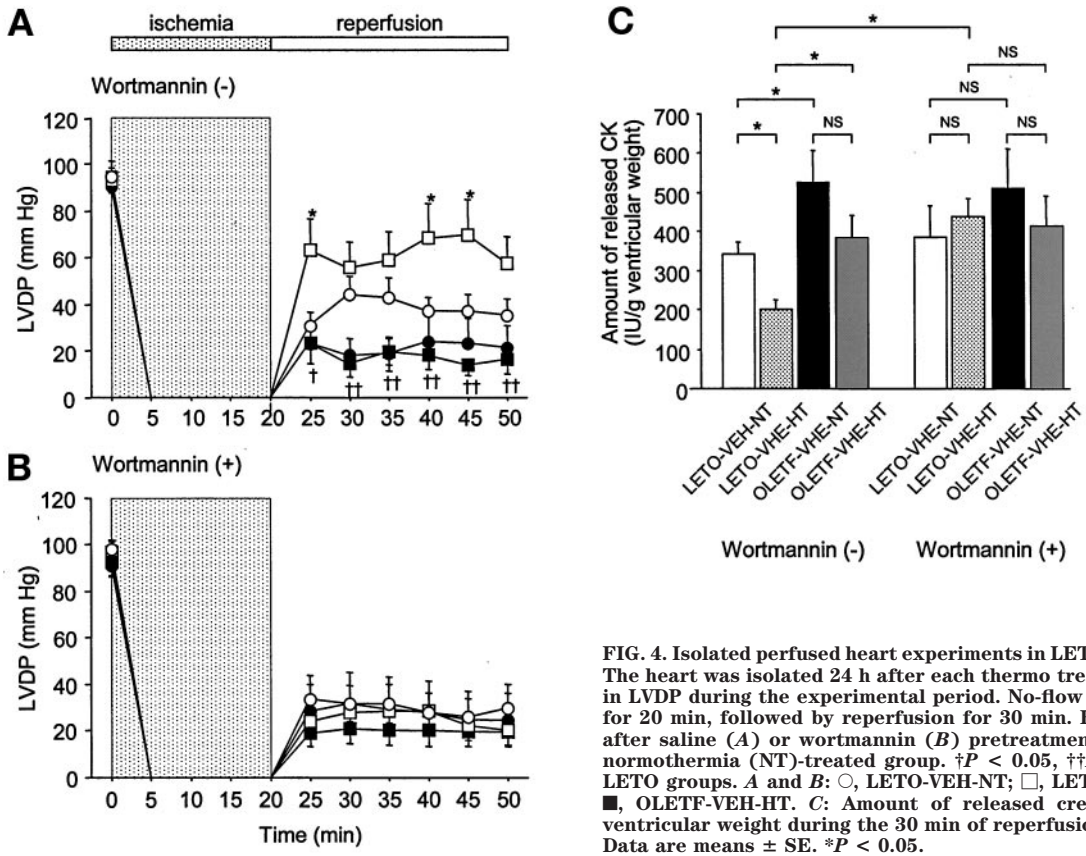

FIG. 4. Isolated perfused heart experiments in LETO-VEH and OLETF-VEH groups. The heart was isolated 24 h after each thermo treatment. *A* and *B*: Serial changes in LVDP during the experimental period. No-flow global ischemia was introduced for 20 min, followed by reperfusion for 30 min. Hyperthermia (HT) was applied after saline (*A*) or wortmannin (*B*) pretreatment. **P* < 0.05 vs. corresponding normothermia (NT)-treated group. †*P* < 0.05, ††*P* < 0.01 vs. the corresponding LETO groups. *A* and *B*: ○, LETO-VEH-NT; □, LETO-VEH-HT; ●, OLETF-VEH-NT; ■, OLETF-VEH-HT. *C*: Amount of released creatine kinase (CK) relative to ventricular weight during the 30 min of reperfusion period. *n* = 7 for each group. Data are means ± SE. **P* < 0.05.

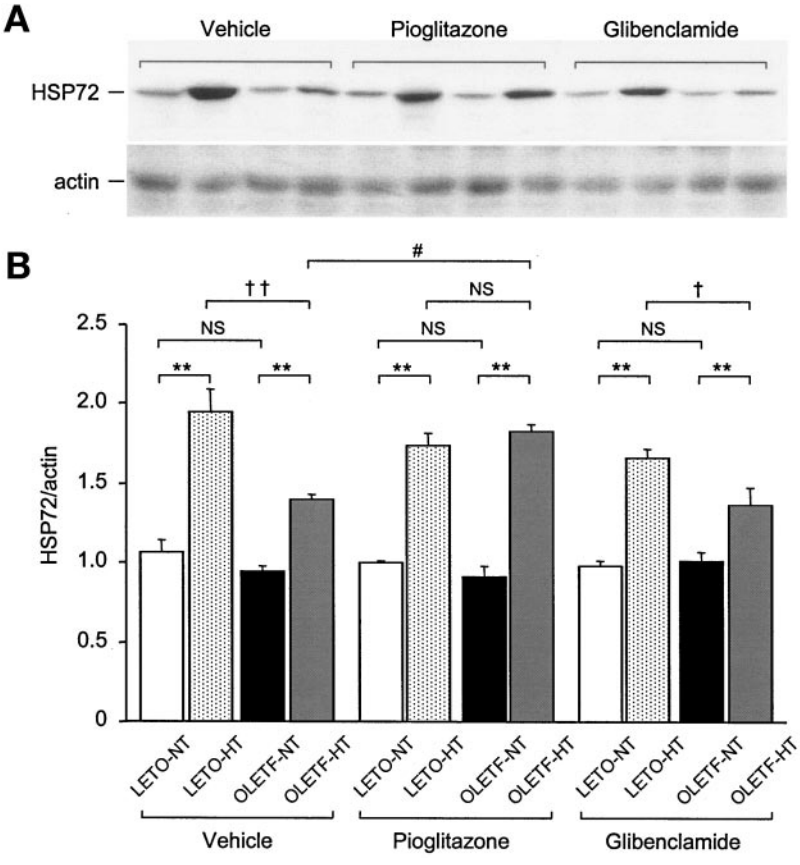

FIG. 5. Effects of pioglitazone or glibenclamide treatment on cardiac HSP72 expression. The heart was isolated 24 h after each thermo treatment. Representative immunoblot bands (*A*) and quantification of the ratio of HSP72 to actin (*B*). *n* = 4 for each group. Data are means ± SE. HT, hyperthermia; NT, normothermia. ***P* < 0.01 vs. corresponding NT-treated groups; †*P* < 0.05, ††*P* < 0.01 vs. corresponding LETO groups; #*P* < 0.05 vs. vehicle-treated group.

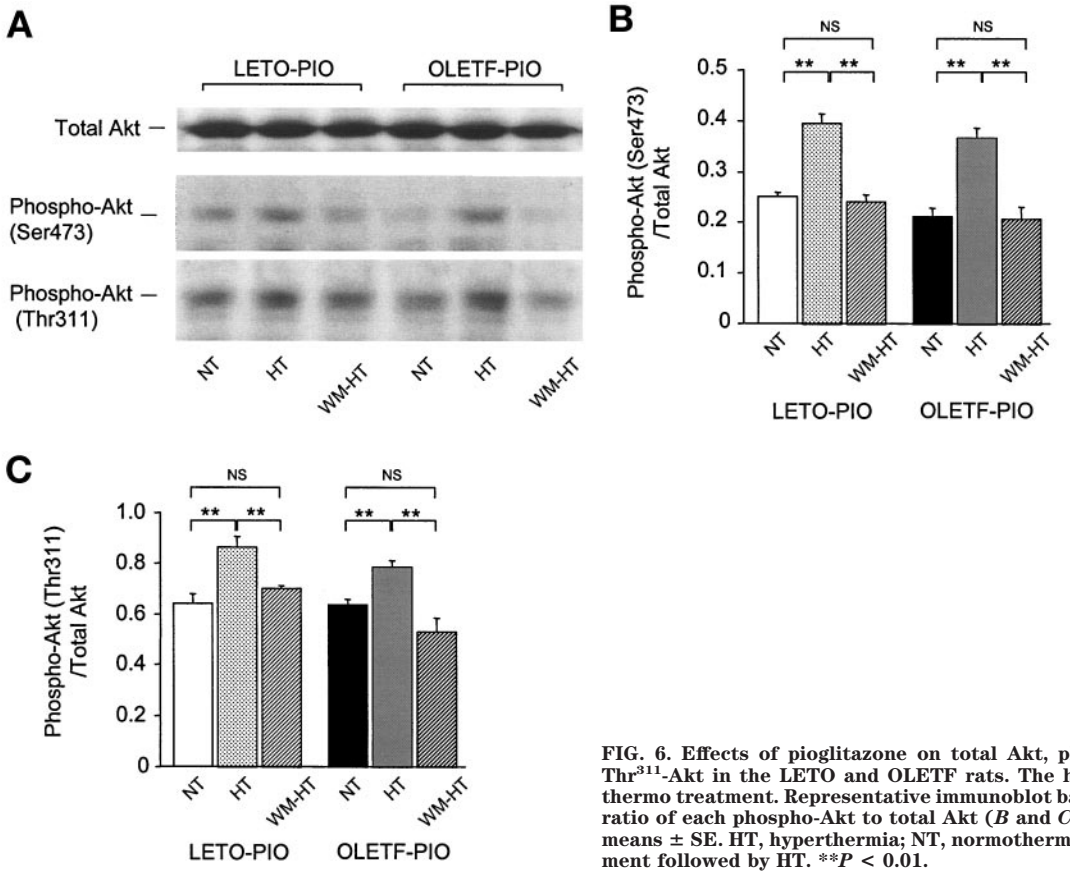

FIG. 6. Effects of pioglitazone on total Akt, phospho-Ser^{473}-Akt, and phospho-Thr^{311}-Akt in the LETO and OLETF rats. The heart was isolated 1 h after each thermo treatment. Representative immunoblot bands (*A*) and quantification of the ratio of each phospho-Akt to total Akt (*B* and *C*). $n = 4$ for each group. Data are means ± SE. HT, hyperthermia; NT, normothermia; WM-HT, wortmannin pretreatment followed by HT. **$P < 0.01$.

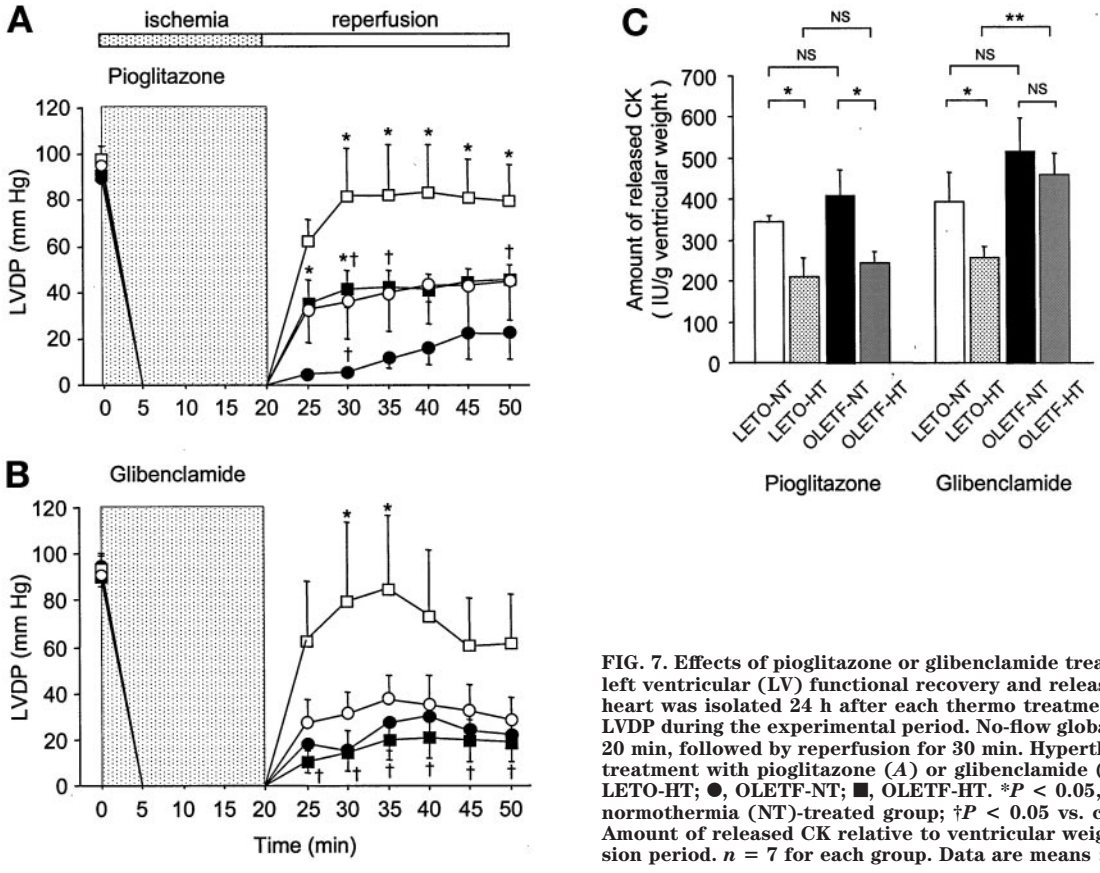

FIG. 7. Effects of pioglitazone or glibenclamide treatment on reperfusion-induced left ventricular (LV) functional recovery and released creatine kinase (CK). The heart was isolated 24 h after each thermo treatment. *A* and *B*: Serial changes in LVDP during the experimental period. No-flow global ischemia was introduced for 20 min, followed by reperfusion for 30 min. Hyperthermia (HT) was applied after treatment with pioglitazone (*A*) or glibenclamide (*B*). *A* and *B*: ○, LETO-NT; □, LETO-HT; ●, OLETF-NT; ■, OLETF-HT. *$P < 0.05$, **$P < 0.01$ vs. corresponding normothermia (NT)-treated group; †$P < 0.05$ vs. corresponding LETO group. *C*: Amount of released CK relative to ventricular weight during the 30-min reperfusion period. $n = 7$ for each group. Data are means ± SE. *$P < 0.05$, **$P < 0.01$.

DISCUSSION

The core findings of the present study are as follows. *1*) In response to hyperthermia, the Akt activation depending on PI3 kinase was necessary for cardiac HSP72 expression. *2*) Hyperthermia-induced Akt activation and HSP72 expression was depressed in OLETF rat hearts. *3*) Pioglitazone but not glibenclamide improved insulin sensitivity in OLETF rats, which was associated with the restoration of Akt activation and HSP72 expression. *4*) In isolated perfused heart experiments, reperfusion-induced left ventricular functional recovery was suppressed in OLETF rat hearts, which was restored by treatment with pioglitazone but not glibenclamide.

In the present study, we used OLETF rats, which have been established as an animal model of type 2 diabetes, characterized by late onset of hyperglycemia (after 20 weeks of age), mild course of diabetes, and conversion to insulin-dependent diabetes after ~40 weeks of age (22,23). In our protocol, 12-week-old rats were introduced to receive oral pioglitazone or glibenclamide for 4 weeks. At 16 weeks, when compared with corresponding control LETO rats, while plasma glucose concentrations were not significantly different, plasma insulin concentrations were higher in OLETF rats. In addition, during OGTT, increases of plasma glucose and insulin concentrations were greater in OLETF rats. Thus, vehicle-treated OLETF rats of 16 weeks of age can be regarded as an insulin-resistant model but not a type 2 diabetic model. As shown in Table 1 and Fig. 1, in OLETF rats, both pioglitazone and glibenclamide suppressed the increase of plasma glucose during OGTT. However, their effects on plasma insulin were strikingly different. While pioglitazone improved insulin sensitivity, glibenclamide rather worsened it. These findings suggest that the differences of our observations between pioglitazone-treated and glibenclamide-treated OLETF rats may be, at least in part, due to differed alteration in insulin sensitivity despite the similar levels of plasma glucose.

Information is very limited with respect to the impact of insulin resistance and type 2 diabetes on HSP72 expression. In the study using human subjects, Kurucz et al. (9) provided the first evidence of decreased expression of the HSP72 mRNA in skeletal muscle from patients with type 2 diabetes, with this reduction being correlated with some markers of insulin resistance. Consistently, Bruce et al. (24) demonstrated that intramuscular HSP72 and heme oxygenase-1 mRNA were reduced in patients with type 2 diabetes. The authors reported the more convinced correlation between a reduction in HSP72 mRNA with insulin resistance (24). However, these clinical studies evaluated the HSP72 mRNA expression only in skeletal muscle and did not provide the underlying mechanism. In this regard, we have focused on PI3 kinase–dependent Akt phosphorylation as a triggering mechanism for hyperthermia-induced HSP72 expression. In fact, we have recently reported that transient activation of Akt in a PI3 kinase–dependent manner is required for hyperthermia-induced HSP72 expression and that streptozotocin (STZ)-induced diabetic rat heart showed an attenuation of this response, resulting in depressed HSP72 expression (19). Because insulin supplementation restored these abnormalities, we proposed insulin deficiency is a predominant etiology for depressed PI3 kinase–dependent Akt activation in response to hyperthermia in STZ-induced diabetic heart (19). It is noteworthy that OLETF rats, characterized by insulin resistance, also showed attenuated PI3 kinase–dependent Akt phosphorylation in response to hyperthermia in the present study. Although it appears difficult to explain the disparity, these two animal models may have common pathogenesis in a sense. The cardiac muscles in STZ-induced diabetic rat cannot utilize insulin due to insulin deficiency while those in OLETF rat cannot effectively utilize insulin because of insulin resistance. This hypothesis may be supported by the combination of our previous (19) and present observations; that is, both insulin supplementation in STZ-induced diabetic rat (19) and improvement in insulin resistance in OLETF rat by pioglitazone in the present study could restore the PI3 kinase–dependent Akt phosphorylation and resultant HSP72 expression.

Thiazolidinediones, including pioglitazone, are synthetic PPAR-γ agonists, acting as insulin sensitizers (14,15). In the last few years, it has been reported numerically that the therapeutic benefits of PPAR-γ agonists may reach far beyond their use as insulin sensitizers (25). It is noteworthy that pioglitazone reportedly leads to increased expression of HSP72 in the gastric mucosa in acetic-acid gastric ulcers in association with promoting ulcer healing (26). Regarding myocardial ischemia/reperfusion injury, chronic troglitazone administration improved left ventricular functional recovery and increased net myocardial lactate uptake, indicating that troglitazone enhances myocardial carbohydrate oxidation (27). It was also reported that rosiglitazone attenuated the increase in caspase-3 activity and apoptotic cell death (28). More recently, using Zucker diabetic fatty (ZDF) rats, Yue et al. (29) reported that oral rosiglitazone administration for 8 days reduced ischemia/reperfusion-induced infarct size and apoptosis, which was in association with an improvement of insulin sensitivity. It is noteworthy that Akt phosphorylation in ZDF rat heart subjected to ischemia/reperfusion was enhanced by rosiglitazone. In their experiments, the Akt phosphorylation might induce cell surviving cascade against apoptosis. Thus, the restoration of Akt phosphorylation in response to hyperthermia observed in pioglitazone-treated OLETF rats may be due to diverse effects derived from the activation of PPAR-γ.

Limitations. There are several reservations in the present study. First, we discussed PI3 kinase–dependent Akt phosphorylation as a predominant signal for hyperthermia-induced HSP72 overexpression. However, in either OLETF or LETO rats, while the pretreatment with wortmannin completely inhibited Akt phosphorylation by hyperthermia (Fig. 3), it resulted in failure to completely suppress the HSP72 expression (Fig. 2). These observations suggest the working of operative mechanisms other than activation of PI3 kinase–dependent Akt activation for hyperthermia-induced HSP72 expression, which should be clarified in a future study. Second, our experiments could not establish whether pioglitazone-induced HSP72 expression improves reperfusion-induced cardiac functional recovery. To answer this issue appropriately, specific inhibition of expression of the HSP72 gene by, for instance, intracoronary injection of antisense HSP72 oligonucleotide, will be required. Third, in the clinical setting, it appears impossible to introduce the patients to temperatures as high as 43°C. It should be determined whether other HSP72 inducers, including GGA (5,6), lead to similar results. Fourth, our experiments were not performed in a single session under the same conditions. For instance, the absolute ratio of phospho-Akt/total Akt that appears in Fig. 3 is difficult to compare with what appears in Fig. 6.

Finally, the amount of released creatine kinase did not correlate well with the LVDP recovery during reperfusion period (Figs. 4 and 7). Especially in hyperthermia-treated OLETF rat hearts, the reperfusion-induced left ventricular functional recovery appeared to be depressed compared with the reduction in released creatine kinase. The finding suggests the complicated functional damage of OLETF rat hearts in response to ischemia/reperfusion, which was not reflected as released creatine kinase.

Conclusions. Our results suggest that PI3 kinase–dependent Akt activation in response to hyperthermia, an essential signal for HSP72 expression, at least in part, is depressed in the heart of insulin-resistant OLETF rats, and the results also suggest that restorations of HSP72 expression and tolerance against ischemia/reperfusion injury with pioglitazone in OLETF heart might be due to a restoration of impaired PI3 kinase–dependent Akt activation in response to hyperthermia.

ACKNOWLEDGMENTS

This study was supported in part by a grant-in-aid 17590756 (T.S.) from the Japanese Ministry of Education, Science and Culture.

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