Acute hyperglycemia exacerbates myocardial ischemia/reperfusion injury and blunts cardioprotective effect of GIK

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Su H, Sun X, Ma H, Zhang H-F, Yu Q-J, Huang C, Wang X-M, Luan R-H, Jia G-L, Wang H-C, Gao F. Acute hyperglycemia exacerbates myocardial ischemia/reperfusion injury and blunts cardioprotective effect of GIK. Am J Physiol Endocrinol Metab 293: E629–E635, 2007. First published May 22, 2007; doi:10.1152/ajpendo.00221.2007.—There is a close association between hyperglycemia and increased risk of mortality after acute myocardial infarction (AMI). However, whether acute hyperglycemia exacerbates myocardial ischemia/reperfusion (MI/R) injury remains unclear. We observed the effects of acute hyperglycemia on M/I/R injury and on the cardioprotective effect of glucose–insulin–potassium (GIK). Male rats were subjected to 30 min of myocardial ischemia and 6 h of reperfusion. Rats were randomly received one of the following treatments (at 4 ml·kg⁻¹·h⁻¹ iv): Vehicle, GIK (GIK during reperfusion; glucose: 200 g/l, insulin: 60 U/l, KCL: 60 mmol/l), HG (high glucose during ischemia; glucose: 500 g/l, GIK + HG (HG during I and GIK during R) or GIK + wortmannin (GIK during R and wortmannin 15 min before R). Blood glucose, plasma insulin concentration and left ventricular pressure (LVP) were monitored throughout the experiments. Hyperglycemia during ischemia not only significantly increased myocardial apoptosis (23.6 ± 1.7% vs. 18.8 ± 1.4%, P < 0.05 vs. vehicle), increased infarct size (IS) (45.6 ± 3.0% vs. 37.6 ± 2.0%, P < 0.05 vs. vehicle), decreased Akt phosphorylation (1.1 ± 0.1 vs. 1.7 ± 0.2% vs. vehicle), and increased GSK-3β phosphorylation (2.8 ± 0.1% fold of vehicle, respectively, P < 0.05 vs. vehicle) following MI/R, but almost completely blocked the cardioprotective effect afforded by GIK, as evidenced by significantly increased apoptotic index (9.1 ± 2.0 vs. 10.3 ± 1.2%, P < 0.01 vs. GIK), increased myocardial IS (39.2 ± 2.8 vs. 27.2 ± 2.1%, P < 0.01 vs. GIK), decreased Akt phosphorylation (1.1 ± 0.1 vs. 1.7 ± 0.2% vs. vehicle, P < 0.01 vs. GIK) and GSK-3β phosphorylation (1.4 ± 0.2 vs. 2.3 ± 0.2%, P < 0.05 vs. GIK). Hyperglycemia significantly exacerbates MI/R injury and blocks the cardioprotective effect afforded by GIK, which is, at least in part, due to hyperglycemia-induced decrease of myocardial Akt activation.

Akt: glucose–insulin–potassium

IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION (AMI), stress hyperglycemia can commonly be observed. An association between hyperglycemia and an increased risk of mortality and poor prognosis after AMI was well noted in patients with or without diabetes (1, 21). Mechanisms for the association between stress hyperglycemia and adverse outcomes after AMI in nondiabetic patients are not well understood. Additional studies showed that acute hyperglycemia in AMI patients without diabetes was independently associated with larger enzymatic infarct size and higher long-term mortality rates after AMI (22). These results suggest that poor outcomes of patients with AMI may be related to hyperglycemia. However, direct evidence to support a causative role of acute hyperglycemia in exacerbating myocardial ischemia/reperfusion (MI/R) injury is not currently available, and the underlying mechanisms remain unidentified.

Glucose–insulin–potassium (GIK) has been applied in AMI for more than 40 years, but the results of some GIK clinical trials are very different and controversial. The meta-analysis of GIK (7) and several randomized high-dose GIK clinical trials [such as ECLA (5) and DIGAMI (17)] suggest a beneficial effect of GIK in AMI patients. However, some clinical trials of GIK showed different results. In the DIGAMI 2 study (18), the primary target of a fasting blood glucose level of 90–126 mg/dl in the insulin group was not achieved in this trial and insulin-glucone oxidation showed no treatment benefits. In the CREATE-ECLA trial (19), relative hyperglycemia occurred in the GIK treatment group and GIK did not improve mortality. As shown in these studies, it seems that GIK (or GI) infusion in the presence of hyperglycemia has no beneficial effect on outcomes. Therefore, it is possible that hyperglycemia obscures the protective effect afforded by GIK.

Our previous studies (9, 25) demonstrated that GIK may play an important role to attenuate MI/R injury in vivo when it was administrated during reperfusion and activation of Akt through the phosphatidylinositol 3-kinase (PI 3-kinase)-dependant mechanism as the central mediator of the protective effect of GIK against MI/R injury. Interestingly, it was demonstrated that hyperglycemia can induce oxidative stress, increase the generation of free radicals and proinflammatory cytokines, further impair activation of Akt, and increase apoptosis in cultured cardiocytes (8). But it is not clear whether acute hyperglycemia impairs the activation of Akt induced by insulin in ischemia/reperfusion (I/R) myocardium in vivo.

Therefore, the objectives of the present study were to 1) determine whether acute hyperglycemia during ischemia exacerbates MI/R injury in vivo, 2) evaluate the hypothesis that acute hyperglycemia blocks GIK-induced myocardial protection in I/R heart, and 3) investigate the mechanism involved.

MATERIALS AND METHODS

Experimental protocol. The experiments were performed in adherence with the National Institutes of Health Guidelines on the Use of Laboratory Animals and were approved by the Fourth Military Medical University Committee on Animal Care. Eighty adult male

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Sprague-Dawley rats were anesthetized through intraperitoneal administration of 30 mg/kg pentobarbital sodium. A microcatheter was inserted into left ventricular through right carotid artery to measure the left ventricular pressure. The artery pressure was measured by right femoral artery intubation. Intravenous infusion was executed through left external jugular vein. Electrocardiogram (ECG) and heart rate (HR) were simultaneously recorded on a polygraph (RM-6200C). Hemodynamic data were continuously monitored on a polygraph and simultaneously digitized by using a computer interfaced with an analog-to-digital converter. Blood samples were drawn from caudal vein before ischemia, 30 min after ischemia, 2 h and 4 h after reperfusion, respectively, to measure blood glucose levels and plasma insulin concentrations. Myocardial ischemia was produced by exteriorizing the heart through a left thoracic incision and placing a 6-0 silk suture and making a slipknot around the left anterior descending coronary artery. After 30 min of ischemia, the slipknot was released, and the myocardium was reperfused for 4 h (for analysis of myocardial infarct size) and 6 h (for quantification of myocardial infarct size).

Rats were randomized to receive one of the following solutions by intravenous infusion at a rate of 4 ml kg⁻¹ h⁻¹: 1) Vehicle: saline throughout the whole ischemia and reperfusion period; 2) GIK: saline during ischemia and GIK (glucose 200 g/l, insulin 60 U/l, KCI 60 mmol/l) during reperfusion; 3) HG: as described previously (6), HG (glucose 500 g/l) during ischemia, saline during reperfusion; 4) GIK + HG: HG during ischemia plus GIK during reperfusion; and 5) GIK + wortmannin: wortmannin, a specific inhibitor that has been demonstrated to be highly selective for PI-3 kinase (15 μg/kg iv 15 min before reperfusion), saline during ischemia and GIK during reperfusion.

**Determination of plasma creatine kinase and lactate dehydrogenase.** Blood samples (1 ml) were drawn at 2 and 4 h after reperfusion, respectively. Plasma creatine kinase (CK) and lactate dehydrogenase (LDH) activities were measured spectrophotometrically (Beckman DU 640) in a blinded manner. All measurements were assayed in duplicate.

Terminal deoxynucleotidyl nick-end labeling assay. Myocardial apoptotic index was analyzed by TUNEL assay as described previously (16). A double-staining technique was used, i.e., TUNEL staining for apoptotic cell nuclei and DAPI staining for all myocardial cell nuclei. TUNEL staining was performed by using an In Situ Cell Death Detection Kit (Roche Molecular Biochemicals) according to the protocol provided by the manufacturer. In brief, cardiomyocytes from at least four slides per block that were randomly selected were evaluated immunohistochemically to determine the number and percentage of cells exhibiting positive staining for apoptosis. For each slide, 10 fields were randomly chosen, and a total of 100 cells per field were counted by using a defined rectangular field area (×20 objective). The index of apoptosis was determined [(no. of apoptotic myocytes/total no. of myocytes counted) × 100%] from a total of 40 fields per heart, and the assays were performed in a blinded manner.

**Quantification of myocardial infarct size.** The myocardial infarct size (IS) was determined by means of a double-staining technique and was analyzed by a digital imaging system described previously (10). At the end of the 6-h reperfusion period, the ligature around the coronary artery was retied, and 1 ml of 2% Evans blue dye was injected into the left ventricular cavity. The dye was circulated and uniformly distributed except in the portion of the heart that was previously perfused by the occluded coronary artery (area-at-risk, AAR). The heart was quickly excised, frozen at −20°C, and sliced into 1-mm-thick sections perpendicular to the long axis of the heart by using a heart slice chamber. Slices were incubated in 1% TTC in phosphate buffer (pH 7.4) at 37°C for 10 min and photographed with a digital camera. Evans’s blue-stained area (area-not-at-risk, ANAR), TTC-stained area (red staining, ischemic but viable tissue), and TTC staining negative area (infarct myocardium) were measured digitally using Image Pro Plus software (Media Cybernetics). AAR was expressed as a percentage of the LV (AAR/LV), and IS was expressed as a percentage of the AAR (IS/AAR).

**Western blot.** Ischemic myocardium tissue samples were lysed with lysis buffer. After sonication, the lysates were centrifuged, proteins were separated by electrophoresis on SDS-PAGE and then transferred onto PVDF (polyvinylidene difluoride)-Plus membrane (Micron Separations). After being blocked with 5% milk, the immunoblots were probed with anti-phospho-(p)Akt and anti-pGSK-3β antibodies (Cell Signaling, Beverly, MA) overnight at 4°C followed by incubation with the corresponding secondary antibodies at room temperature for 1 h. The blots were visualized with ECL-plus reagent. pAkt and pGSK-3β immunoblots were then stripped with strip buffer at 50°C for 30 min and reblotted for total Akt and GSK-3β (anti-Akt and anti-GSK-3β antibodies were from Cell Signaling).

**Statistical analysis.** All values are presented as means ± SE. Differences were compared by ANOVA or Student’s t-test where appropriate. Probabilities of <0.05 were considered to be statistically significant. All of the statistical tests were performed with the GraphPad Prism software version 4.0 (GraphPad Software, San Diego, CA).

**RESULTS**

**Plasma insulin and blood glucose concentrations.** Plasma insulin concentration was not changed during the whole MI/R procedure in vehicle group, whereas GIK treatment during reperfusion significantly increased insulin concentration. With the infusion of HG during ischemia, plasma insulin increased at 30 min after ischemia and gradually decreased during reperfusion. Interestingly, wortmannin plus GIK significantly increased the plasma insulin by 27.3 and 21.1% at 2 and 4 h after reperfusion, respectively (both $P < 0.05$ compared with GIK alone), suggesting that both acute hyperglycemia and wortmannin provoke insulin secretion.

Blood glucose concentrations did not change in the vehicle and GIK group but increased in the HG and GIK + HG groups during administration of supplemental intravenous HG (Table 1).

**Systemic hemodynamics.** No significant differences in systemic hemodynamics were observed among all groups under baseline conditions. Elevated ST segment and T wave occurred immediately when the blood flow of the left anterior descending coronary artery was blocked. There were no significant differences in heart rate and blood pressure among all groups during ischemia or reperfusion period, although blood pressure decreased in all groups during ischemia. The instantaneous first derivation of left ventricle pressure ($±LVdP/dt_{max}$) also decreased in all groups during ischemia, but no significant differences existed among all groups. Situations were different during the reperfusion period (Fig. 1). $±LVdP/dt_{max}$ increased by 11.2 and 9.9%, respectively, in GIK group and decreased by 10.5 and 10.4%, respectively, in HG group in relevant to vehicle group ($P < 0.05$) after 2 h of reperfusion. After 4 h of reperfusion, $±LVdP/dt_{max}$ increased by 11.5 and 10.7%, respectively, in the GIK group and decreased by 11.8 and 9.2%, respectively, in the HG group compared with the vehicle group ($P < 0.05$). At 4 h after reperfusion, $±LVdP/dt_{max}$ decreased by 11.4 and 10.1%, respectively, in the GIK + HG group compared with the GIK group ($P < 0.05$).

Previous studies have demonstrated that activation of PI-3 kinase-Akt signaling contributes to protection of insulin on I/R myocardium; however, whether this survival signaling pathway is also involved in the effect of hyperglycemia still remains elusive. To investigate the mechanisms underlying the effect of hyperglycemia on the myocardial functions following...
MI/R, an additional group was studied in which animals were pretreated with wortmannin, a selective PI 3-kinase inhibitor, 15 min before reperfusion. As seen in Fig. 1, although treatment with wortmannin itself had no significant effect on MI/R rats in (+)LdP/d(t max (data not shown), this treatment completely blocked the functional protective effects of GIK (P < 0.05 vs. GIK group). Moreover, there was no difference in cardiac function between the GIK + wortmannin group and the GIK + HG group (Fig. 1). These data suggest that HG during ischemia reduced cardiac function and blocked the cardioprotective effect of GIK, possibly through an Akt-mediated pathway.

Myocardial infarct size. To determine whether hyperglycemia might aggravate myocardial injury, the effects of GIK, HG, GIK + HG, and GIK + wortmannin on myocardial infarct size (IS) were determined (Fig. 2). There were no significant differences in AAR/LV among all groups (data not shown). Consistent with our previous results (9), treatment with GIK significantly decreased IS (27.2 ± 2.1 vs. 37.6 ± 2.0% in the

Table 1. Plasma insulin and blood glucose in anesthetized rats subjected to myocardial ischemia and reperfusion receiving different treatments

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline</th>
<th>I 30 min</th>
<th>R 2 h</th>
<th>R 4 h</th>
</tr>
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<tbody>
<tr>
<td>Plasma insulin (mU/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>18.1 ± 1.0</td>
<td>15.4 ± 1.4</td>
<td>19.6 ± 1.1</td>
<td>19.7 ± 1.2</td>
</tr>
<tr>
<td>GIK</td>
<td>15.7 ± 1.3</td>
<td>17.6 ± 1.2</td>
<td>69.5 ± 5.5†</td>
<td>63.0 ± 4.7†</td>
</tr>
<tr>
<td>HG</td>
<td>20.4 ± 1.0</td>
<td>66.8 ± 5.9§</td>
<td>54.8 ± 3.6†</td>
<td>46.5 ± 4.6‡</td>
</tr>
<tr>
<td>GIK + HG</td>
<td>20.1 ± 1.1</td>
<td>63.3 ± 4.9§</td>
<td>91.0 ± 6.8§</td>
<td>100.7 ± 8.1§</td>
</tr>
<tr>
<td>GIK + W</td>
<td>18.6 ± 1.2</td>
<td>19.0 ± 1.4</td>
<td>88.5 ± 5.8‡</td>
<td>76.3 ± 7.7‡</td>
</tr>
</tbody>
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Blood glucose (mmol/l) |          |          |       |       |
| Vehicle | 4.7 ± 0.3 | 4.7 ± 0.2 | 4.9 ± 0.4 | 5.2 ± 0.3 |
| GIK     | 5.1 ± 0.2 | 4.9 ± 0.2 | 5.4 ± 0.4 | 6.1 ± 0.4 |
| HG      | 4.7 ± 0.2 | 18.0 ± 0.4* | 8.6 ± 0.6* | 6.6 ± 0.4* |
| GIK + HG| 4.8 ± 0.3 | 18.4 ± 0.5+‡ | 9.4 ± 0.6*‡ | 7.9 ± 0.5+‡ |
| GIK + W | 5.2 ± 0.4 | 5.0 ± 0.2 | 6.5 ± 0.6 | 7.1 ± 0.5 |

Values are means ± SE; n = 16. GIK, glucose-insulin-potassium; HG, hyperglycemia; W, wortmannin (a PI 3-kinase inhibitor); I 30 min; 30 min after the beginning of ischemia; R 2 h, 2 h after onset of reperfusion; R 4 h, 4 h after onset of reperfusion. *P < 0.05; †P < 0.01 vs. vehicle; §P < 0.05; ¶P < 0.01 vs. GIK.

Fig. 1. Hyperglycemia during ischemia reduced cardiac contractile function and blocked the cardioprotective effect of GIK. A: +LdP/d(t max, the instantaneous first derivation of left ventricle pressure. B: −LdP/d(t max, GIK, glucose-insulin-potassium; HG, hyperglycemia; W, wortmannin (a PI 3-kinase inhibitor); I, ischemia; R, reperfusion. Values presented are means ± SE; n = 16. *P < 0.05 vs. vehicle; †P < 0.05 vs. GIK.

Fig. 2. Hyperglycemia during ischemia increased myocardial infarct size and blocked the cardioprotective effect of GIK. TTC-negative staining portions (infarct) and TTC-positive staining portions within ischemic/reperfused regions [area at risk (AAR)] were digitally measured, and results were expressed as %infarct area (INF) relative to AAR. Values presented are means ± SE; n = 8. *P < 0.05, **P < 0.01 vs. vehicle; ##P < 0.01 vs. GIK.
crease of plasma CK and LDH activities by 21.1 and 24.5% before ischemia partly blunted the GIK-induced decrease of IS (both P < 0.05 vs. GIK group), whereas it made no difference compared with those rats treated with GIK + HG (Fig. 3). These results suggest that hyperglycemia during ischemia may inhibit the GIK-induced decrease in cardiomyocyte necrosis following MI/R in vivo.

Myocardial apoptosis. A significant number of TUNEL-positive cells (18.8 ± 1.4%) were observed in myocardial tissue from hearts subjected to ischemia and reperfusion in the vehicle group. Consistent with our previous study, administration of HG during reperfusion exerted a significant antiapoptotic effect by reduced TUNEL-positive staining (10.3 ± 1.2%, P < 0.01 vs. vehicle group). HG exacerbated myocardial apoptotic death as evidenced by increased TUNEL-positive staining (23.6 ± 1.7%, P < 0.05 vs. vehicle group). Interestingly, TUNEL-positive staining in myocytes increased by 85% over the GIK in the GIK + HG group, and the antiapoptotic effects of GIK were abolished. In addition, pretreatment with wortmannin before reperfusion partly blocked the GIK-induced decrease of apoptosis (17.6 ± 1.6%, P < 0.05 vs. GIK group), whereas it made no difference compared with those rats treated with GIK + HG (Fig. 3). These data showed that hyperglycemia not only increased myocardial apoptosis but also abolished the antiapoptotic effect of GIK in rats subjected to MI/R.

Akt phosphorylation and activation. In our previous studies (9), we demonstrated that GIK exerts a cardioprotective effect via activation of Akt in a PI3-kinase-dependent fashion in the I/R myocardium in vivo. However, it is unknown whether hyperglycemia abolishes the beneficial effect of GIK by inhibiting Akt phosphorylation induced by insulin, the key protective component in GIK. To determine the mechanisms underlying the effect of hyperglycemia on I/R myocardium, we measured the Akt expression and phosphorylation by Western blotting in I/R myocardium in rats receiving different treatments. As shown in Fig. 4, there were no significant differences in total Akt expression between all groups. Treatment with GIK resulted in a 1.7-fold increase in Akt phosphorylation compared with vehicle (P < 0.01 vs. vehicle), whereas Akt phosphorylation was decreased in the HG group (0.5-fold of vehicle, P < 0.05 vs. vehicle). Interestingly, when MI/R rats were infused with HG during ischemia and GIK during reperfusion, Akt phosphorylation induced by insulin was markedly suppressed (1.1-fold of vehicle, P < 0.01 vs. GIK group). Pretreatment with wortmannin before reperfusion inhibited the GIK-induced increase of Akt phosphorylation (0.9-fold of vehicle, P < 0.01 vs. GIK group), whereas it made no difference compared with those rats treated with GIK + HG (Fig. 5). These results suggested that in vivo administration with HG during ischemia inhibited Akt phosphorylation and abolished PI 3-kinase-induced Akt phosphorylation by GIK.

GSK-3β is one of the downstream targets of Akt, and we measured the level of GSK-3β phosphorylation to further determine the Akt activity. As shown in Fig. 6, consistent with the levels of pAkt, GIK and HG resulted in a 2.3-fold increase and a 0.6-fold decrease in GSK-3β phosphorylation in myocardium in MI/R rats (P < 0.01 and P < 0.05, respectively). Interestingly, when MI/R rats were infused with HG during ischemia and GIK during reperfusion, insulin-induced GSK-3β phosphorylation was remarkably suppressed (1.4-fold of vehicle, P < 0.05 vs. GIK group). As anticipated, wortmannin pretreatment before reperfusion almost abolished the GSK-3β phosphorylation.
phosphorylation in GIK-treated rats (1.1-fold of vehicle, $P < 0.05$ vs. GIK group). There was no difference in total GSK-3 in all groups.

These results indicate that hyperglycemia during ischemia reduced GIK-induced Akt phosphorylation and activation in a PI 3-kinase-dependent fashion in myocardium in rats subjected to MI/R.

DISCUSSION

We have made several novel observations in our present experiment. First, we have demonstrated that hyperglycemia during ischemia significantly exacerbated myocardial injury as evidenced by significantly enlarged infarct size, increased myocardial apoptosis, and worse cardiac function following MI/R. Second, hyperglycemia during ischemia blunted the cardioprotective effect afforded by GIK. Third, we have provided evidence that impairment of myocardial activation of Akt is a likely mechanism that accounts for the findings that hyperglycemia exacerbates MI/R injury and blocks the cardioprotective effect of GIK.

Whether acute hyperglycemia alters the extent of MI/R injury is controversial. Some studies showed that hyperglycemia can increase myocardial IS and myocyte apoptosis, exaggerate LV failure, and decrease survival after MI/R by increasing inflammation and oxidative stress and abolishes ischemic preconditioning (4, 13, 14, 20). In contrast, other studies demonstrated that hyperglycemia protected against ischemia-induced myocardial damage, decreased the myocardial IS and the number of apoptotic myocytes, and improved the recovery of heart function after MI/R (3, 24). In the present study, rats were subjected to 30 min of ischemia and 4 h of reperfusion, and severe myocardial injury was observed. Compared with the rats subjected to MI/R and receiving vehicle, the rats administered HG during the ischemia procedure, in which the blood glucose level increased from 4.7 ± 0.2 mmol/l at baseline to 18.0 ± 0.4 mmol/l 30 min after ischemia and 6.6 ± 0.4 mmol/l 4 h after reperfusion, and the latter two blood
glucose values were both higher than those of corresponding time points in the vehicle group, showed larger infarct size, more apoptosis, and worse cardiac functional change. Recent studies showed that patients with AMI exhibit raised blood glucose concentrations, which have been correlated with increases in mortality after AMI in patients with and without diabetes, indicating that increased plasma glucose, rather than the presence of diabetes, increases the risk of complications due to AMI (1, 2). Together with all these results, our present data suggest that hyperglycemia during ischemia significantly exacerbates myocardial injury and thus may worsen the prognosis of the subjects with AMI.

Although several studies suggested that GIK protects the myocardium and preserves heart function during ischemia and reperfusion, the results of some clinical trials are controversial (5, 19). In the ECLA clinical trial, a statistically significant reduction in mortality and a consistent trend toward fewer in-hospital events in the GIK group were observed, and there was no statistically significant difference in serum glucose between the GIK group and the control group (5). In contrast, in the CREATE-ECLA study it seems that high-dose GIK infusion had a neutral effect on mortality, cardiac arrest, and cardiogenic shock in patients with acute STEMI (ST segment elevation myocardial infarction), and there was an increase in serum glucose concentration in the GIK infusion group compared with the control group at 6 and 24 h after treatment (19). Therefore, we hypothesized that the higher serum glucose level in the GIK infusion group might blunt the benefits of GIK/insulin. To test the aforementioned hypothesis, we treated the MI/R rats with HG during the ischemia procedure and with GIK during the reperfusion procedure. Consistent with our previous as well as others' studies, administration of GIK at the onset of reperfusion reduced myocardial infarct size and improved cardiac functional recovery following MI/R in vivo. Most importantly, we found for the first time that, compared with the GIK group, the rats receiving HG during ischemia and GIK during reperfusion showed larger infarct size, more apoptosis, and worse cardiac functional change, in which the blood glucose level was 18.4 ± 0.5 mmol/l at 30 min after ischemia and 7.9 ± 0.5 mmol/l at 4 h after reperfusion and both were higher than those in the GIK group. In addition, in one of our recent studies (26), we demonstrated that treatment of dogs in vivo with insulin alone at the dose that reduced blood glucose to a clinically tolerable level exerted significant cardioprotective effects that were comparable to that seen in the GIK-treated group. Together with the results of previous clinical trials, these data suggest that hyperglycemia during ischemia blunts the cardioprotective effect afforded by GIK and thus may attenuate the beneficial effect of GIK on mortality in patients with AMI. The ECLA data showed that, regarding the impact of GIK in patients within 12 h of symptom onset, the earlier the solution is infused, the more evident is the effect obtained. In the CREATE-ECLA study, 83% of patients had reperfusion therapy at a median time of 3.85 h after symptom onset. But randomization to GIK or control groups occurred almost 1 h later (median 4.7 h postsymptom onset); then GIK was started mostly “within the next hour.” This late, often post, reperfusion, administration minimized GIK’s potency to reduce ischemic injury, maybe because of failure in controlling the blood glucose level induced by the oxidative stress.

Hyperglycemia may harm myocardium through multiple pathways. It leads to increased production of reactive oxygen species (ROS) through the hexosamine biosynthetic pathway (12, 15). In cultured ventricular myocytes incubated in a medium containing high concentrations of glucose, free radical generation (especially ROS production) and proinflammatory cytokine concentrations were drastically raised, and the number of dead and apoptotic myocytes markedly increased (8). Therefore, it is likely that the increased oxidative stress induced by hyperglycemia may be the primary mechanism responsible for enhanced ischemia/reperfusion injury observed in animals treated with HG. Furthermore, using a human ventricular heart cell model of simulated ischemia-reperfusion, Verma et al. (23) demonstrated that cellular injury was greater in human ventricular heart cells subjected to hyperglycemic conditions.

Recent evidence indicates that increased production of ROS caused by hyperglycemia of diabetes leads to serine phosphorylation of IRS-1, which impairs its ability to bind and activate PI 3-kinase, finally diminishing activation of downstream kinases Akt (11). In our previous study, we demonstrated that administration of insulin activates Akt through the PI 3-kinase-dependent mechanism and that this signaling system plays an important role in the cardioprotective effect of GIK in MI/R rats. These results suggest that insulin and hyperglycemia have mutual influences on the PI 3-kinase-Akt signaling pathway, but whether hyperglycemia abolishes the cardioprotection of GIK/insulin by impairing the activation of Akt in vivo during ischemia/reperfusion is still unknown. Our present data showed that there were no significant differences in total Akt expression among all groups, but hyperglycemia during ischemia inhibited the phosphorylation of Akt and GSK-3β, one of downstream targets of Akt, not only in I/R myocardium treated with vehicle but also in I/R myocardium treated with GIK in vivo. Interestingly, pretreatment with wortmannin before reperfusion not only inhibited the GIK-induced increase of Akt activation but also blunted the cardioprotective effects of GIK. Most importantly, there was no significant difference in the effects of wortmannin and hyperglycemia on GIK treatment, suggesting that HG blocks insulin’s cardioprotection, at least partly, by blocking insulin-induced Akt activation. To further confirm this finding, we performed an additional experiment in which the rats were subjected to 30 min of ischemia and 6 h of reperfusion and received GIK, HG, and wortmannin and the myocardial infarction and Akt phosphorylation were observed. As anticipated, HG could not further aggravate the inhibiting effects of wortmannin on the GIK-induced infarct size reduction and Akt activation (data not shown). These results suggest that hyperglycemia exerts adverse effects on ischemic myocardial injury and cardioprotection of GIK at least partly by inhibiting the PI 3-kinase-Akt signaling.

Our data on plasma insulin also suggested that either acute hyperglycemia or wortmannin treatment provokes robust insulin secretion. Although both acute hyperglycemia and wortmannin increased the insulin concentration in blood, these two treatments significantly blunted the insulin-induced PI 3-kinase-Akt signaling pathway, thus blunting the beneficial effects of GIK and aggravated MI/R tissue injury. However, in the present study, similar blood glucose concentrations were observed in the GIK and GIK + wortmannin groups. This was possibly due to the increased insulin that partly compensated...
for the inhibitory effect of wortmannin on insulin-mediated PI 3-kinase and Akt activation, which resulted in no difference in blood glucose concentrations between the GIK and GIK + wortmannin groups.

In summary, in the present study we have demonstrated that hyperglycemia during ischemia significantly exacerbates MI/R injury and blunts the cardioprotective effect afforded by GIK. Impairment of myocardial activation of PI 3-kinase-Akt signaling is a likely mechanism that accounts for the findings that hyperglycemia blunts the cardioprotection effect afforded by GIK.

GRANTS

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