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ARTICLE

Comparison of two sulfonylureas with high and low myocardial K_{ATP} channel affinity on myocardial infarct size and metabolism in a rat model of type 2 diabetes

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Abstract
Aims/hypothesis Sulfonylureas (SUs) may impair outcome in patients with acute coronary syndrome. Most experimental studies of the myocardial effects of SU treatment are performed in non-diabetic models. We compared the effect of two widely used SUs, glibenclamide (gb) and gliclazide (gc), with high and low myocardial K_{ATP} channel affinity, respectively, at therapeutic concentrations on infarct size, left ventricular (LV) function and myocardial glycogen, lactate and alanine content before and after ischaemia/reperfusion (I/R).
Methods Non-diabetic Wistar and diabetic Goto–Kakizaki rat hearts were investigated in a Langendorff preparation. Gb (0.1 μmol/l) and gc (1.0 μmol/l) were administrated throughout the study. Infarct size was evaluated after 120 min of reperfusion. Myocardial metabolite content was measured before and after ischaemia.
Results Infarct size was smaller in diabetic hearts than in non-diabetic hearts (0.33±0.03 vs 0.51±0.05, $p<0.05$). Gb increased infarct size (0.54±0.04 vs 0.33±0.03, $p<0.05$) and reduced post-ischaemic LV developed pressure (60±3 vs 76±3 mmHg, $p<0.05$) and coronary flow (4.9±0.5 vs 7.1±0.4 ml min^{-1} g^{-1}, $p<0.05$) in gb-treated diabetic rats compared with untreated diabetic rats. On comparing gb-treated diabetic rats with untreated diabetic rats, glycogen content was reduced before (9.1±0.6 vs 13.6±1.0 nmol/mg wet weight, $p<0.01$) and after ischaemia (0.9±0.2 vs 1.8±0.2 nmol/mg wet weight, $p<0.05$), and lactate (4.8±0.4 vs 3.2±0.3 nmol/mg wet weight, $p<0.01$) and alanine (1.38±0.12 vs 0.96±0.09 nmol/mg wet weight, $p<0.05$) contents were increased during reperfusion. Gc-treatment of diabetic and non-diabetic rats did not affect any of the measured variables.
Conclusions/interpretations Gb, but not gc, exacerbates I/R injury and deteriorates LV function in diabetic hearts. These effects of gb on diabetic hearts may be due to detrimental effects on myocardial carbohydrate metabolism.

Keywords Glibenclamide · Gliclazide · Glycogen · Ischemia · Lactate · Reperfusion

Abbreviations

ALV	Area of left ventricle
CF	Coronary flow
gb	Glibenclamide
gc	Gliclazide
GK	Goto–Kakizaki
HR	Heart rate
I/R	Ischaemia/reperfusion
IZ	Infarct zone
K_{ATP}	ATP-sensitive potassium channel

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LV	Left ventricular
LVP	Left ventricular pressure
LVDP	Left ventricular developed pressure
RPP	Rate pressure product
RZ	Risk zone
SU	Sulfonylurea
SUR	Sulfonylurea receptor
Wis	Wistar

Introduction

Sulfonylureas (SUs) are widely used in the treatment of patients with type 2 diabetes mellitus. The drugs stimulate insulin secretion from pancreatic beta cells through closure of the ATP-sensitive potassium (K_{ATP}) channel. However, K_{ATP} channels are also expressed in extrapancreatic tissues such as cardiac and vascular smooth muscle cells. Treatment with SUs has been suspected of worsening clinical outcome in patients with acute coronary syndrome [1, 2], but the cardiovascular side effects do not seem to be uniform among all SUs [3, 4].

The K_{ATP} channel is an octameric complex of an inwardly rectifying potassium channel, Kir6.2 or Kir6.1, and an SU receptor (SUR) [5]. Kir6.2 is used as the pore-forming subunit in most tissues, whereas the type of SUR is more variable [5]. SUs differ in affinity and specificity for the SUR subtypes present in various tissues [6, 7]. While glibenclamide (gb) has moderate tissue specificity, gliclazide (gc) has high affinity for SUR1, the pancreatic receptor, but low affinity for cardiac SUR2A and vascular SUR2B [7, 8]. Accordingly gb, but not gc, is reported to abolish ischaemic preconditioning of the heart [9]. Moreover, genetic modulation of SUR1 subunits influences myocardial infarct size in mice hearts, indicating a role for cardiomyocyte survival during myocardial stress [10].

K_{ATP} channels open when intracellular ATP concentration decreases, and cellular changes in K_{ATP} channel activity are considered secondary to intracellular metabolic alterations. In addition, we [11] and others [12–15] have previously demonstrated changes in myocardial metabolism including alterations in the rate of ATP hydrolysis, the cardiac pool of adenine nucleotides and glycogen content secondary to K_{ATP} channel modulation. However, most studies of the effects of K_{ATP} channel modulation have been performed in non-diabetic animals.

The aim of the present study was to compare the effects of gb and gc in therapeutic concentrations on myocardial infarct size and left ventricular (LV) function, and to compare their effects on myocardial glycogen, lactate and alanine content in type 2 diabetic and non-diabetic animal models.

Methods

Animals and study design

The rats were handled according to national guidelines in Denmark and the Principles of Laboratory Animal Care (NIH publication No. 86-23, revised 1985) were followed. Male Wistar (Wis) rats and Goto–Kakizaki (GK) rats (Taconic M&B, Eiby, Denmark) were purchased at 6–8 weeks of age and studied at the age of 16 weeks. Animals were provided with access to food and water ad libitum in a room maintained at 23°C and 50% humidity with a 12 h light–dark cycle. The animals were divided into six groups: (1) control; (2) control plus gb (0.1 μmol/l); (3) control plus gc (1 μmol/l); (4) type 2 diabetes; (5) type 2 diabetes plus gb (0.1 μmol/l); and (6) type 2 diabetes plus gc (1 μmol/l).

Isolated heart preparation

Rats were anaesthetised using midazolam (0.25 mg/kg bodyweight intramuscularly; Dormicum, Roche, Basle, Switzerland) and fluanisone (0.5 mg/kg body weight intramuscularly; Hypnorm, Janssen-Cilag, Beerse, Belgium). A tracheotomy was performed and the animal was connected to a ventilator (Zoovent, Newport Pagnell, UK). Subsequently, a thoracotomy was performed and the heart dissected free from surrounding tissues. A bolus of 1,000 IU/kg heparin (Leo Pharma, Copenhagen, Denmark) was given through the femoral vein. The heart was cannulated in situ, mounted in a Langendorff apparatus and perfused retrogradely with Krebs–Henseleit solution at a pressure of 80 mmHg as previously described [16, 17]. All hearts were subjected to 40 min preischaemic stabilisation.

Experimental protocols

The study consisted of two sub-studies (Fig. 1). The first studied regional no-flow ischaemia (Fig. 1a; infarct study, $n=10$ in each group). A snare was placed around the left main coronary artery 2 mm from its origin. The hearts were exposed to regional no-flow ischaemia for 45 min and a 120 min reperfusion. In the second study (Fig. 1b; metabolic study, $n=10$ in each group), hearts were exposed to 30 min of global no-flow ischaemia by shutting off perfusion followed by a 60 min reperfusion. Myocardial metabolite content was measured before and after 30 min of ischaemia and after reperfusion (Fig. 1). Gb (0.1 μmol/l) and gc (1 μmol/l) were administered after 20 min of stabilisation in drug-treated groups. The concentrations were chosen from reported serum concentrations of gb [18] and gc [19] in humans and estimated protein binding [20].

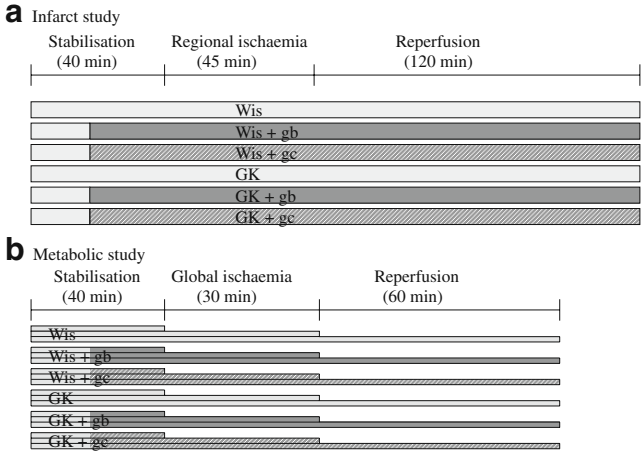

Fig. 1 The effects of gb (0.1 μmol/l) and gc (1.0 μmol/l) were investigated in (**a**) an infarct sub-study using regional ischaemia and (**b**) a metabolic sub-study using global ischaemia. Drugs were administrated as indicated by grey and hatched bars. In the metabolic sub-study myocardial glycogen, lactate alanine and glutamate were measured after stabilisation, ischaemia and reperfusion as indicated by the length of the bars (each horizontal bar represents one group). Control, light grey bar; gb, dark grey bar; gc, hatched bar

Evaluation of myocardial infarction

While still in the Langendorff model, the risk zone (RZ) was defined by ligating the left coronary artery at the site of the occlusion and infusing 3 ml of a 1% solution of Evans Blue (Sigma, St Louis, MO, USA), leaving the RZ unstained. The hearts were frozen for 15 min at –20°C and sliced into six to eight 1.5 mm slices. At 37°C and at a pH of 7.4, the slices were immersed in 1% 2,3,5-triphenyltetrazolium chloride (Sigma) for 10 min to delineate areas of infarction. The hearts were then stored overnight in 2 ml of Lillies solution (4% formaldehyde buffer; VWR International, Rødovre, Denmark) to enhance the colour contrasts. The next day, each slice was weighed and photographed with a digital camera (Nikon Coolpix 5700; Nikon Corporation, Tokyo, Japan). The RZ, the area of the left ventricle (ALV) and the infarct zone (IZ) were assessed by computer planimetry (Analysis [Build 776]; Soft Imaging Systems GmbH, Münster, Germany). The infarct size (IZ/RZ) and the area at risk (RZ/ALV), by weight, were then calculated. All analyses were performed in a blinded manner.

Evaluation of LV function

A latex balloon (Hugo Sachs Elektronik, March-Hugstetten, Germany) was placed in the left ventricle through an incision in the left atrium and held in place by the mitral valve. The volume of the balloon was adjusted to obtain an end diastolic pressure of 7 mmHg. A pressure transducer (Baxter Cardiovascular Group, Irvine, CA, USA) was connected to the latex balloon, allowing recording of the left ventricular pressure (LVP). The analogue signal from the transducer was converted (AD-converter, MP100 system; BioPAC Systems, Goleta, CA, USA) and stored on a computer. Coronary flow (CF) was monitored continuously by a flowmeter (Transonic, Maastricht, the Netherlands). The left ventricular developed pressure (LVDP) was calculated as $P_{LV,\ systolic} - P_{LV.diastolic}$ and the rate pressure product (RPP) as the LVDP×heart rate (HR).

Assessment of myocardial glycogen, lactate, glutamate and alanine content

In the global no-flow model, hearts were freeze-clamped within 2 s in liquid nitrogen (–196°C) using pre-cooled Wollenberger clamps and stored at –80°C. Glycogen was determined by the filter-paper technique described by Sølling and Esmann [21] and modified by Bøtker et al. [22]. Lactate, glutamate and alanine were measured from the same homogenates using enzymatic analyses and spectrophotometric detection [23, 24].

Assessment of biochemical variables and in vivo blood pressure

Serum levels of glucose, insulin, cholesterol, triacylglycerol and NEFA were measured after 8 h of fasting. In a subset of animals, blood pressure was measured by tail plethysmography.

Statistics and calculations

All values are expressed as the mean ± SEM. Groups were compared using ANOVA or two-way ANOVA repeated measures for comparison on haemodynamic data. When appropriate, exploratory post hoc tests were performed. $p<0.05$ was considered statistically significant. SPSS version 10 (SPSS, Chicago, IL, USA) was used for statistical calculations.

Results

Infarct study

Myocardial infarction Infarct size (IZ/RZ) was 30% smaller ($p<0.05$) in diabetic hearts than in non-diabetic hearts (Fig. 2a). We found no differences in infarct size between untreated, gb- and gc-treated non-diabetic animals. Gb treatment increased myocardial infarct size by 60% ($p<0.05$) in diabetic hearts compared with untreated diabetic hearts and in diabetic hearts compared with gc-

treated diabetic hearts ($p<0.01$). Gc treatment of diabetic animals did not influence infarct size compared with untreated diabetic animals. The area at risk (RZ/ALV) did not differ between the groups ($p=0.89$; Fig. 2b).

Left ventricular function and coronary flow Gb and gc treatment did not influence LVDP, RPP or CF before or after ischaemia, or after reperfusion in non-diabetic animals (Table 1). In diabetic animals LVDP ($p<0.01$) and RPP ($p<0.05$) were significantly reduced during ischaemia compared with non-diabetic controls. While gb did not influence LVDP, RPP or CF in diabetic animals before or after ischaemia, all three variables were significantly reduced in gb-treated diabetic animals compared with untreated ($p<0.05$) and gc-treated diabetic animals during reperfusion (LVDP: $p<0.01$; RPP: $p<0.05$; CF: $p<0.05$).

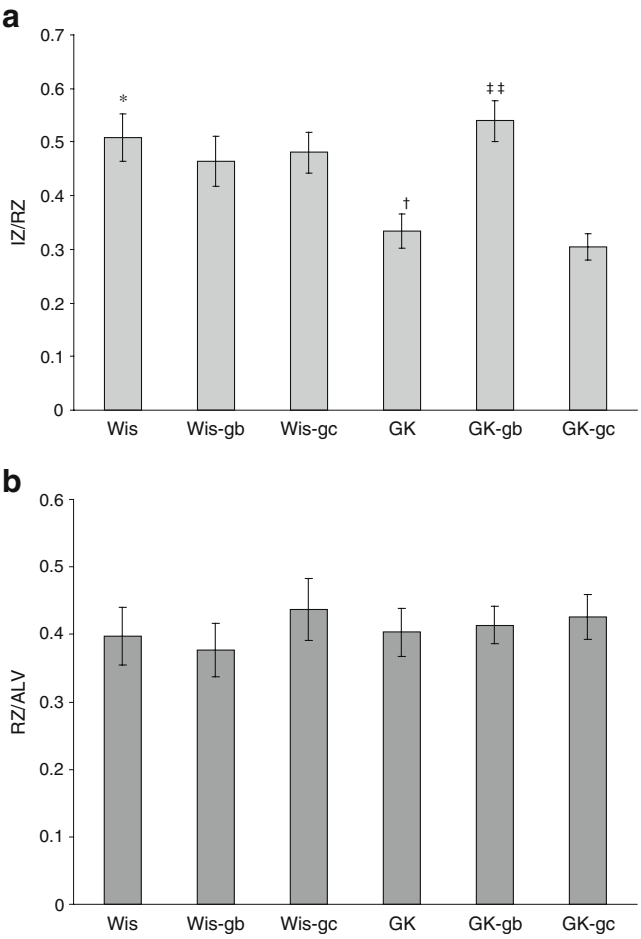

Fig. 2 **a** Myocardial infarct size (IZ/RZ) after 45 min of regional ischaemia and 120 min of reperfusion. Infarct size was smaller in diabetic hearts compared with control hearts. Gb increased infarct size in diabetic animals compared with untreated and gc-treated diabetic rats. **b** Area at risk (RZ/ALV) did not differ between groups.*$p<0.05$ vs GK; †$p<0.05$ vs GK-gb; ‡‡$p<0.01$ vs GK-gc. $n=10$ in each group

Gc did not influence LVDP, RPP or CF in diabetic animals.

Metabolic study

Myocardial glycogen and lactate content Myocardial glycogen content before ischaemia was significantly increased (30%, $p<0.05$) in diabetic compared with non-diabetic animals (Fig. 3a). No differences in myocardial glycogen or lactate content were found between diabetic and non-diabetic animals after ischaemia or reperfusion (Fig. 3b). In diabetic animals gb treatment reduced myocardial glycogen content before (35%, $p<0.01$) and after (50%, $p<0.05$) ischaemia and after reperfusion (45%, $p<0.05$) and increased lactate content before ischaemia (65%, $p<0.05$) and after reperfusion (30%, $p<0.01$) compared with untreated diabetic controls. Compared with gc-treated diabetic animals, gb treatment reduced myocardial glycogen content before ($p<0.01$) and after ($p<0.01$) ischaemia and after reperfusion ($p<0.05$). Myocardial lactate content was increased before ischaemia ($p<0.01$) and after reperfusion ($p<0.05$) in gb-treated animals compared with gc-treated diabetic animals. Gb did not significantly influence myocardial glycogen and lactate content in non-diabetic animals. However, there was a trend of reduced myocardial glycogen content before ($p=0.14$) and after ($p=0.09$) ischaemia in gb-treated non-diabetic hearts. Gc treatment of diabetic and non-diabetic animals did not influence myocardial glycogen or lactate content, respectively, compared with controls.

Myocardial alanine and glutamate content Myocardial alanine content did not differ between diabetic and non-diabetic animals before ischaemia ($p=0.71$; Fig. 4a). Gb treatment increased myocardial alanine content during reperfusion compared with untreated ($p<0.05$) and gc-treated ($p<0.05$) diabetic animals. Myocardial glutamate content was increased before ischaemia in diabetic compared with non-diabetic animals ($p<0.01$; Fig. 4b). Neither gb nor gc treatment influenced myocardial glutamate content in diabetic or non-diabetic groups.

Biochemical variables, blood pressure and body weight

GK rats showed hyperglycaemia, hyperinsulinaemia, hypercholesterolaemia and reduced NEFA and triacylglycerol levels compared with non-diabetic controls (Table 2). Blood pressure did not differ in GK rats and controls (Table 2). Body weight was higher in non-diabetic animals compared with GK rats ($p<0.01$; Table 2). However, body weight did not differ between groups of non-diabetic animals (Wis: 454.5±16.1 g; Wis-gb: 481.7±

Table 1 Haemodynamics in perfused rat hearts after 40 min of stabilisation, 45 min of regional ischaemia and 120 min reperfusion

Group	LVDP (mmHg)			RPP (mmHg/BPM)/1,000			CF (ml min^{-1} g^{-1})		
	Stab	Isc	Rep	Stab	Isc	Rep	Stab	Isc	Rep
Wis	121±4	81±4**	75±4	25.3±1.7	15.2±0.9*	13.6±1.1	9.5±0.5	6.8±0.4	7.1±0.5
Wis-gb	115±6	74±3	78±3	25.8±1.3	13.9±1.0	14.7±1.0	9.2±0.6	6.7±0.5	7.0±0.5
Wis-gc	118±5	81±4	75±4	27.3±1.3	15.0±0.9	14.8±0.7	9.9±0.5	7.1±0.6	6.6±0.4
GK	119±6	62±3	76±3†	27.9±1.2	11.1±0.7	13.7±0.7†	9.7±0.4	7.0±0.4	7.1±0.4†
GK-gb	110±5	58±4	60±3‡‡	23.8±2.0	10.3±0.7	10.2±0.7‡	9.1±0.7	7.1±0.5	4.9±0.5‡
GK-gc	120±6	64±3	81±3	25.2±2.1	11.5±0.6	14.2±0.8	9.5±0.5	7.1±0.3	7.2±0.6

BPM, beats per minute; Isc, ischaemia; Rep, reperfusion; Stab, stabilisation

*p<0.05 vs GK; **p<0.01 vs GK; †p<0.05 vs GK-gb; ‡p<0.05 vs GK-gc, ‡‡p<0.01 vs GK-gc

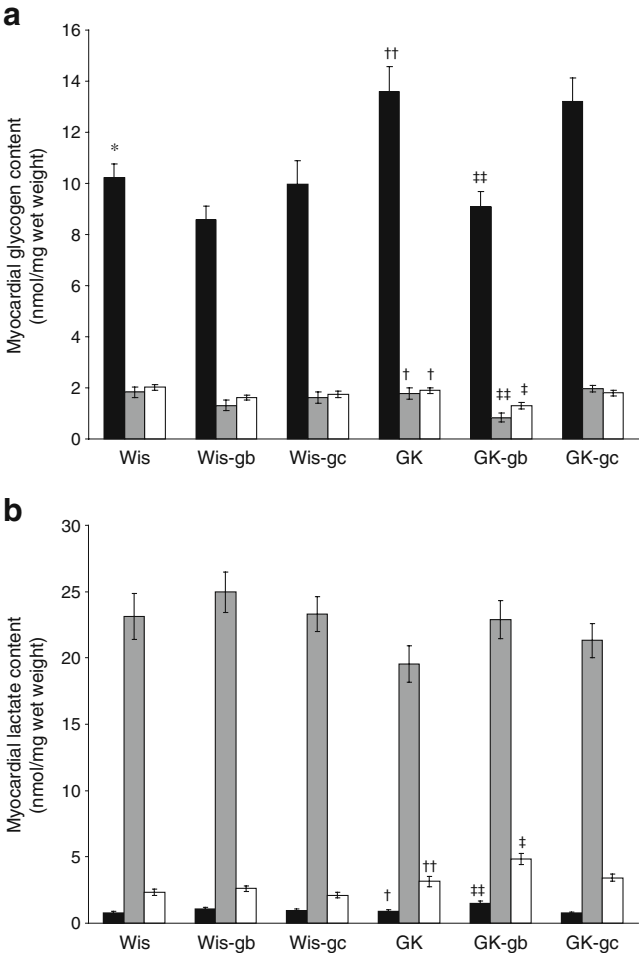

Fig. 3 **a** Myocardial glycogen and **b** lactate content (nmol/mg wet weight) after 40 min stabilisation (black bars), 30 min ischaemia (grey bars) and 60 min reperfusion (white bars). *p<0.05 vs GK; †p<0.05 vs GK-gb; ††p<0.01 vs GK-gb; ‡p<0.05 vs GK-gc; ‡‡p<0.01 vs GK-gc. n=10 in each group

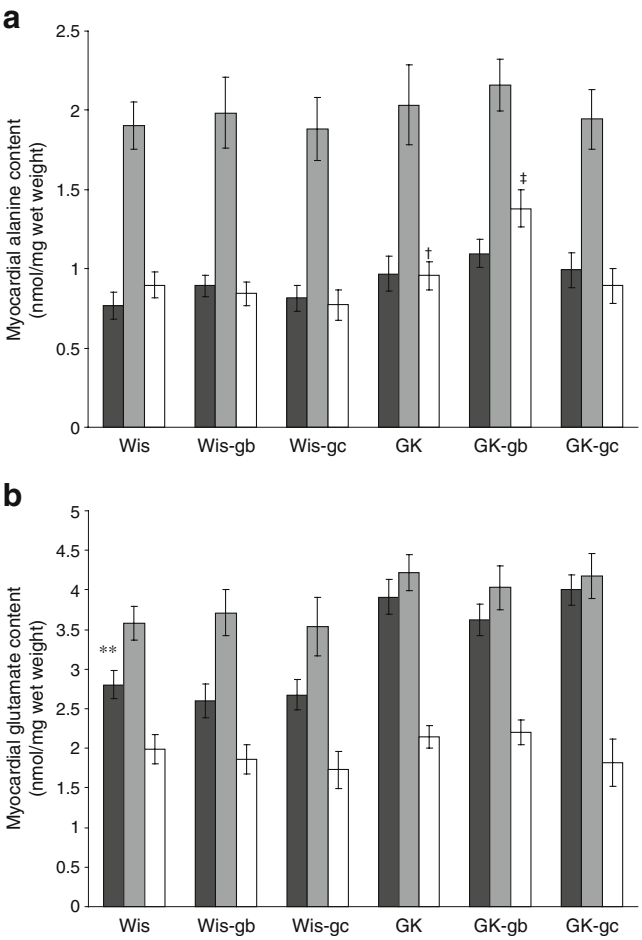

Fig. 4 **a** Myocardial alanine content (nmol/mg wet weight) after 40 min stabilisation (black bars), 30 min ischaemia (grey bars) and 60 min reperfusion (white bars). Gb increased myocardial alanine content during reperfusion compared with both untreated and diabetic rats. **b** Myocardial glutamate content was increased in untreated diabetic compared with untreated non-diabetic animals before ischaemia. **p<0.01 vs GK; †p<0.05 vs GK-gb; ‡p<0.05 vs GK-gc. n=8–10 in each group

Table 2 Clinical and metabolic variables in Wis and GK rats at termination of the study

Variable	Wis	GK
Body weight (g)	466.3±10.3	409.8±9.3**
Fasting glucose (mmol/l)	4.6±0.2	8.3±0.3***
P-insulin (pmol/l)	315±19	383±22
P-NEFA (mmol/l)	0.85±0.05	0.64±0.03**
P-tri (mmol/l)	1.79±0.20	0.99±0.10**
P-chol (mmol/l)	1.65±0.13	1.98±0.05*
MAP (mmHg)	114±6	123±5

chol, cholesterol; MAP, mean arterial pressure; P-, plasma concentration; tri, triacylglycerol

*$p<0.05$ vs Wis; **$p<0.01$ vs Wis; ***$p<0.001$ vs Wis

15.5 g; Wis-gc: 462.9±21.5 g; $p=0.55$) or GK rats (GK: 410.1±21.6 g; GK-gb: 416.8±10.6 g; GK-gc: 402.4±16.7 g; $p=0.84$).

Discussion

We extended previous studies of the influence of SUs on myocardial protection from non-diabetic to diabetic animals and demonstrated that gb and gc in therapeutic concentrations have different influences on myocardial infarct size and function in a model of type 2 diabetes. Gb, but not gc, increased myocardial infarct size, depressed post-ischaemic LV function and reduced myocardial glycogen content in type 2 diabetic animal models. Our findings may explain some of the discrepancies in clinical outcome in diabetic patients with acute coronary syndrome treated with SUs.

Gb and gc do not influence myocardial infarct size in an in vivo non-diabetic rat model [9]. Gc has no influence on cardioprotection by ischaemic preconditioning, but protection was inhibited by gb [9]. We extended these findings by showing that neither gb nor gc influenced myocardial infarct size in non-diabetic animals, whereas gb exacerbates infarct size in diabetic rats. Because the metabolic environment at the time of ischaemia and reperfusion was identical in all groups, the effect of gb on infarct size in diabetic animals cannot be explained by differences in metabolic control. The different responses to gb and gc may rather be related to myocardial differences caused by diabetes. One potential mechanism could be the high glycogen reserves in diabetic rats.

We confirmed that preischaemic myocardial glycogen content is increased in diabetic hearts [25]. High glycogen reserves protect from ischaemic damage and result in smaller infarcts in the diabetic rat heart; this is because they help to maintain energy supply via anaerobic glycolysis during ischaemia. A decrease in myocardial glycogen content is associated with increased infarct size in gb-treated diabetic hearts. Gb treatment in supratherapeutic doses reduces preischaemic myocardial glycogen content in non-diabetic hearts [11]. In the present study we found a similar tendency using a therapeutic dosage, but we did not find any effect on myocardial infarct size. Consequently, the preischaemic myocardial glycogen levels may be of less importance in non-diabetic than in diabetic hearts. An explanation may be that gb impairs myocardial mitochondrial function, but this is unmasked only in the diabetic heart, because gb adds to the deficit in mitochondrial oxidative capacity that generally accompanies diabetes.

Accumulating evidence indicates that type 2 diabetes is accompanied by mitochondrial dysfunction [26] that diminishes glucose oxidation and reduces mitochondrial oxygen efficiency [27]. Gb influences mitochondrial K_{ATP} channels and interferes with mitochondrial bioenergetics in renal tubular cells [28, 29]. In the present study, myocardial alanine content was increased in gb-treated diabetic animals during reperfusion. Alanine, by transamination with glutamate and aspartate being the two main amino group donors, and lactate are the end-products of glycolysis. The increased myocardial content of alanine and lactate during reperfusion may reflect reduced mitochondrial glucose oxidation in gb-treated diabetic animals. L-glutamate is an important metabolite to maintain and restore mitochondrial function as it is a substrate for complex I of the respiratory chain, participates in the malate–aspartate shuttle and serves as an anaplerotic metabolite for replenishment of citric acid cycle metabolites after ischaemia and hypoxia [30–32]. In accordance with the results of the present study, we have previously reported a decreased level of the mitochondrial EAAT-1 (excitatory amino acid transporter) glutamate transporter and increased myocardial glutamate content before ischaemia in hearts from diabetic rats [33]. Increased myocardial glutamate content in the non-ischaemic diabetic hearts may be a compensatory mechanism secondary to a decreased level of mitochondrial glutamate transporters in order to sustain mitochondrial function. In further support of mitochondrial dysfunction in diabetic hearts, glutamate affords cardioprotection, which involves the malate–aspartate shuttle [34], and an increased glutamate concentration is required to elicit protection against ischaemia/reperfusion (IR) injury in diabetic compared with non-diabetic animals [33]. As the modulation of mitochondrial K_{ATP} channels by gc and gb is different [29, 35] gc treatment did not influence cardiac amino acid or mitochondrial metabolism. Hence, we propose that a diabetic mitochondrial dysfunction becomes unmasked during reperfusion in gb-treated diabetic animals.

Gb increased myocardial lactate content in diabetic hearts before ischaemia and after reperfusion. We also observed a decreased CF during reperfusion in gb-treated

diabetic hearts. Gb has low tissue specificity, including affinity for vascular SUR2B receptors, and may modulate myocardial blood flow such that reduced perfusion may compromise metabolism. On the other hand, the reduced flow may also reflect preserved autoregulation and a consequence of impaired haemodynamic function. The reduced flow is rather reflecting preserved autoregulation and a consequence of impaired haemodynamic function. Gb-induced impairment of mitochondrial function results in increased anaerobic metabolism with higher lactate and lower glycogen, which persists as long as the drug is present, i.e. before, during and after ischaemia. Thereby, gb abolishes the 'protective' effect associated with high glycogen, resulting in greater damage during ischaemia, which causes poorer function after ischaemia, i.e. during reperfusion.

Limitations to the present study are mainly related to the isolated perfused rat heart model. Using an ex vivo model may explain our findings of smaller infarct size in GK compared with Wis rats, which is in contrast to results obtained with in vivo models [36, 37]. Animals were subjected to acute SU treatment in the Langendorff model and not to in vivo long-term oral treatment, which may influence the effects observed here. Similar to our findings long-term, gb treatment [38] in contrast to gc [39] is also reported to have detrimental effects after I/R. With regard to clinical relevance, increased tissue glycogen concentrations are typically found in diabetic rat hearts, whereas tissue glycogen content is reduced rather than increased in diabetic humans.

In conclusion, high glycogen reserves protect from ischaemic damage and result in smaller infarcts in the diabetic rat heart. Gb-induced impairment of mitochondrial function is unmasked only in the diabetic heart because gb adds to the deficit in mitochondrial oxidative capacity that generally accompanies diabetes. Gb-induced impairment of mitochondrial function results in increased anaerobic metabolism and increased myocardial ischaemic damage in diabetes.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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