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Glibenclamide attenuates ischemia-induced acidosis and loss of cardiac function in rats

Roger J. Legtenberg [a], Ralph J.F. Houston [a,b], Arend Heerschap [c], Berend Oeseburg [a,1], Paul Smits [d,e,*]

[a] Department of Physiology 237, University Medical Center Nijmegen, P.O. Box 9101, NL-6500 HB, Nijmegen, The Netherlands
[b] Department of Anesthesiology, University Medical Center Utrecht, P.O. Box 85500, 3508 GA, Utrecht, The Netherlands
[c] Department of Radiology, University Medical Center Nijmegen, P.O. Box 9101, 6500 HB, Nijmegen, The Netherlands
[d] Department of Pharmacology-Toxicology, University Medical Center Nijmegen, P.O. Box 9101, 6500 HB, Nijmegen, The Netherlands
[e] Department of Internal Medicine, University Medical Center Nijmegen, P.O. Box 9101, 6500 HB, Nijmegen, The Netherlands

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Abstract

Previous research has shown that the sulfonylurea derivative glibenclamide may improve post-ischemic cardiac functional recovery. Although K_{ATP} channel blockade is a possible explanation for this observation, alternative mechanisms exist. Therefore, we simultaneously recorded cardiac function and the intracellular concentration of ATP, phosphocreatine, Pi and pH before and after ischemia in the presence of glibenclamide or vehicle. ^{31}Phophorus magnetic resonance (MS) spectroscopy on erythrocyte-perfused, isolated working rat hearts was performed. Glibenclamide 4 μmol l^{-1} or vehicle alone was tested (both $n=5$). The following protocol was used: 8 min performance assessment, 10 min drug treatment, 12 min global ischemia, 20 min reperfusion with drug treatment and 8 min functional recovery assessment. Compared with vehicle, glibenclamide significantly decreased coronary blood flow ($59.5 \pm 7.0\%$ vs. $94.3 \pm 1.3\%$, $P=0.008$), ischemia-induced cardiac functional loss ($7.4 \pm 1.3\%$ vs. $18.8 \pm 3.3\%$; $P=0.019$) as well as the ichemia-induced intracellular acidosis (6.75 ± 0.01 vs. 6.43 ± 0.03 for vehicle, $P=0.03$).

In conclusion, glibenclamide is able to reduce the myocardial functional loss after ischemia while preserving pH but not ATP levels during ischemia. This suggests that the beneficial response to glibenclamide is probably not the result of myocardial K_{ATP} channel blockade, but may be explained by inhibition of glycolysis. © 2002 Published by Elsevier Science B.V.

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1. Introduction

Sulfonylurea derivatives, e.g. glibenclamide, are still the corner stone in the treatment of type 2 diabetes mellitus. The blood glucose lowering effect of these drugs is achieved by closing the so-called ATP-sensitive K^+ (K_{ATP}) channels in the pancreatic β cells. This subsequently results in depolarization of the membrane and opening of voltage-gated Ca^{2+} channels. The resulting Ca^{2+} influx ultimately leads to insulin secretion by the pancreatic β cells (Gerich, 1989; Groop, 1992). Interestingly, K_{ATP} channels have also been discovered in the myocardium, and it has been shown that sulfonylurea derivatives are able to interact with these K_{ATP} channels (Noma, 1983). During ischemia, myocardial K_{ATP} channels open due to a decrease of the ATP/ADP ratio, resulting in K^+ efflux which in turn results in hyperpolarization of the myocardial membrane. The subsequent shortening of the action potential preserves energy utilization and in this way the opening of myocardial K_{ATP} channels acts as an endogenous protective mechanism of the heart (Coetzee, 1992; Nichols et al., 1991; Noma, 1983).

Based on this endogenous role of myocardial K_{ATP} channels during ischemia, it is logical that blocking these channels with sulfonylurea derivatives does have an influence on the outcome of an ischemic insult, which has also been shown in several experimental models of ischemia. Glibenclamide may increase the post-ischemic infarct size

* Corresponding author. Tel.: +31-24-3613674; fax: +31-24-3540535.
E-mail address: P.Smits@farm.kun.nl (P. Smits).
[1] Berend Oeseburg sadly died on December 30, 2000.

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and abolish the ischemic preconditioning-induced reduction in post-ischemic infarct size (Gross and Auchampach, 1992; Munch-Ellingsen et al., 1996; Qian et al., 1996; Rohmann et al., 1994; Thornton et al., 1993). This may be explained by counteracting of the above-described endogenous protective mechanism by glibenclamide, resulting in increased energy usage and therefore increased necrotic tissue. On the other hand, it has been shown that glibenclamide improves post-ischemic cardiac function (Ali et al., 1993; Docherty et al., 1997; Legtenberg et al., 2001; Schaffer et al., 1985; Tosaki and Hellegouarch, 1994). This improvement of post-ischemic cardiac function may be explained by the positive inotropic effects of the higher intracellular Ca^{2+} levels due to blockade of the K_{ATP} channel.

However, other mechanisms for glibenclamide-induced improvements of post-ischemic cardiac function can also be active. It has been shown that glibenclamide accelerates depletion of ATP levels and attenuates intracellular acidosis during ischemia, indicating an inhibitory effect on glycolysis (Docherty et al., 1997; Schaffer et al., 1985). Another possibility may be that the uncoupling effect of glibenclamide on mitochondrial oxidative phosphorylation is responsible for the effects of glibenclamide on post-ischemic cardiac function (Minners et al., 2000; Szewczyk et al., 1997).

The goal of the present study was to investigate whether the effects of glibenclamide on post-ischemic cardiac function can be related to intracellular ATP measurements, and so determine whether glibenclamide has an effect on the ischemia-induced ATP depletion.

To study this, we used our isolated, perfused working rat heart model. We perfused the hearts using an erythrocyte-enriched perfusate to ensure adequate oxygen supply to the heart (Olders et al., 1990b; Podesser et al., 1999) and to adequately interpret possible vasoactive effects of glibenclamide. A mild global ischemic insult was chosen to simulate a situation of myocardial stunning post-ischemically. The effects of glibenclamide on intracellular ATP, phosphocreatine, Pi and pH levels were monitored throughout the whole experimental protocol using ^{31}P-magnetic resonance (MR) spectroscopy.

2. Materials and methods

2.1. Reagent and experimental groups

Glibenclamide was purchased from Sigma-Aldrich, Zwijndrecht, The Netherlands. In total, 10 animals were used in two experimental groups; vehicle ($n=5$) and 4 μmol l^{-1} ($n=5$). In a previous study, 4 μmol l^{-1} glibenclamide was shown to be cardioprotective in our model (Legtenberg et al., 2001). Concentrated drug solutions were infused into the system by a syringe with a computer-controlled pump (1:10). The vehicle composition in the syringe was as follows: 1 mmol l^{-1} NaOH, 0.4% dimethylsulfoxide (DMSO), 0.8% NaCl and 1.5% bovine serum albumin (fraction V, ICN Biomedicals, Zoetermeer, The Netherlands). The drug concentration was the free non-protein bound concentration (measured by high-performance liquid chromatography (HPLC) analysis following equilibrium analysis (Russel et al., 1998)). Glibenclamide is highly bound to protein (97.5%), and therefore we used the free unbound concentrations. So, the ''free'' concentration of 4 μmol l^{-1} glibenclamide was achieved using a ''total'' concentration of 160 μmol l^{-1}.

2.2. Animal model

A detailed description of the perfusion set-up has been published elsewhere (Olders et al., 1990a), also using magnetic resonance spectroscopy (Houston et al., 1997). Briefly, a male Wistar rat (400 g nominal weight, 4–6 months old) was anesthetized with diethyl ether, the heart excised via thoracotomy and placed in ice-cold buffer. The aorta was cannulated and Langendorff perfusion with buffer was started within 8–10 min after heart removal. Subsequently, the left atrium was cannulated, and after checking for leaks the system was switched to working configuration with erythrocyte suspension. Fluid columns were used to maintain the preload pressure at 2 kPa and the afterload pressure at 13 kPa.

The buffer (95% O_2 and 5% CO_2) and erythrocyte suspension (18% O_2, 8% CO_2 and the rest N_2) were equilibrated using membrane oxygenators. The composition of the buffer solution was as follows: NaCl 118, $CaCl_2$ 3.0, KCl 4.7, Na_2HCO_3 25, $MgSO_4$ 1.2, KH_2PO_4 1.2, Na_2EDTA 0.5, Glucose 11.1 mmol l^{-1}. The erythrocyte suspension was prepared by washing heparinized bovine blood three times with physiological saline. For the erythrocyte suspension albumin was added to the same buffer solution to 1.5% followed by adding washed bovine erythrocytes to a hematocrit of 0.25. The free Ca^{2+} concentration in this composition was 1.6 mmol l^{-1} as determined by blood gas analysis. The temperature of the heart and perfusate were maintained throughout the experiment at normothermia of a rat (38±1 °C). In the magnet, the temperature of the heart (in air) was maintained using a humidified system and temperature was measured inside the heart with Luxtron® fiber-optic probes (Houston et al., 1998).

2.3. Experimental protocol

After an initial 15–30 min stabilization period, which included the time required for tuning the magnetic resonance probe and shimming of the magnet, baseline cardiac performance was assessed for 8 min with a preload pressure of 2 kPa, holding the afterload pressure constant at 13 kPa (baseline period). Next, drug was infused for 10 min in Langendorff mode at a constant pressure of 13 kPa (pre-ischemic period), followed by 12 min of normothermic global ischemia, achieved by clamping the aorta line.

Thereafter, reperfusion was initiated in Langendorff mode for 20 min, again with infusion of drug (post-ischemic period). Then the set-up was switched back to working mode. Finally, cardiac performance was assessed for the second time in the same way as before (recovery period). Erythrocyte suspension perfusate was used throughout. In Fig. 1, a graphical representation of the coronary blood flow during the whole protocol is shown, illustrating the protocol used. Cardiac performance was assessed by measuring cardiac output (aortic blood flow+coronary blood flow against constant pressure). All the hemodynamic data presented in the Results section are based on the following data points in the different periods unless otherwise stated: baseline period—8th min, pre-ischemic period—10th min, post-ischemic period—20th min, and recovery period—1st min. Aortic blood flow was measured using an ultrasound flow probe (Transonic Systems, Ithaca, NY, USA) and coronary blood flow was measured by collecting and weighing the perfusate dripping off the heart. The coronary blood flow data collected during the pre-ischemic, post-ischemic and recovery periods were normalized to the baseline coronary blood flow data. Functional loss was calculated by dividing the reduction in left ventricular output (aortic flow+coronary blood flow) in the recovery period by the left ventricular output in the baseline period.

2.4. Magnetic resonance spectroscopy

Magnetic resonance spectroscopy experiments were performed using a 7.0-T magnet (Magnex Scientific, Abingdon, England) interfaced to a S.M.I.S. spectrometer (Surrey Medical Imaging Systems, Surrey, England) operating at 300.22 MHz for ^{1}H and at 121.53 MHz for ^{31}P. The horizontal magnet is equipped with a 150 mT/m shielded gradient set and has a free bore size of 120 mm. A solenoid coil was used with a diameter of 20 mm. Field inhomogeneity was adjusted by shimming on the water (^{1}H) signal from the sample yielding line widths of 50–70 Hz. ^{31}P magnetic resonance spectra were acquired using a simple pulse and acquire sequence with a conventional hard 90° radio frequency pulse (=60 μs) with a repetition time of 4 s. The pulse sequence also generated trigger pulses to slightly over-pace the heart (360 bpm) such that acquisition immediately preceded a pacing pulse to minimize movement artifacts. Thirty-two free induction decays were acquired and averaged resulting in a time resolution of 2 min, which gave an adequate signal-to-noise ratio for frequency domain analysis. The sweep width was 10 kHz and 512 data points were collected. The spectra obtained during the 8-min baseline and recovery periods (four time points per period, 128 free induction decays in total) were averaged. Also the 10-min pre-ischemic period spectra (five time points, 160 free induction decays in total), and the last 14 min of the post-ischemic period (seven time points, 224 free induction decays in total) were averaged. During ischemia and the first 6 min of reperfusion, 2 min spectra (32 free induction decays in total) were used.

Signals in ^{31}P magnetic resonance spectra were quantitated by iterative fitting of the time-domain signal with the variable projection method and with appropriate prior knowledge for the ATP-multiplets (Van den Boogaart et al., 1995; Van der Veen et al., 1988) using MRUI software (version 99.7). All the peak areas were obtained by fitting the spectral lines to Gaussian line shapes. All peaks were analyzed relatively to an external reference of methylene diphosphonic acid (resonating at 16 ppm), which is posi-

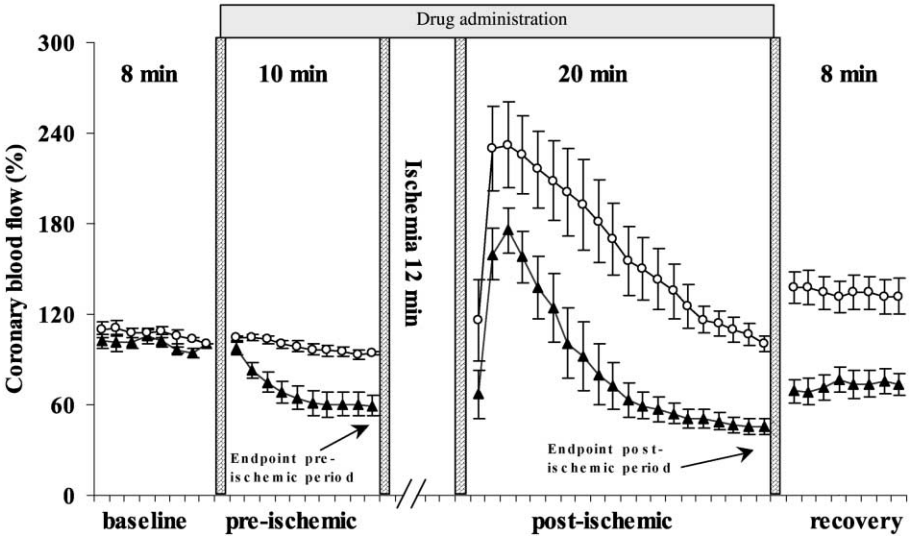

Fig. 1. Graphical representation of the relative coronary blood flow throughout the whole experimental protocol (baseline, pre-ischemic, post-ischemic and recovery periods) and the effects of vehicle (○) and 4 μmol l^{-1} glibenclamide (▲) on this. Results are presented as mean±standard error of the mean (S.E.M.).

tioned adjacent to the heart in the coil. The ATP content was determined from the β-ATP intensity. Its baseline value was used as a reference for the phosphocreatine and Pi levels. The chemical shift of the phosphocreatine-peak was set to 0 ppm and the pH was calculated from the shift between phosphocreatine and Pi as described previously (Jacobus et al., 1978).

2.5. Statistical analysis

The hemodynamic results are presented as mean±standstandard error of the mean (S.E.M.). Differences in coronary blood flow and cardiac functional loss were statistically tested using Student's *t*-test. Differences in metabolic profiles were tested in different sections during the experiment. These sections are: baseline period (8 min—one time point), pre-ischemic period (10 min—one time point), ischemic period (12 min—six time points), post-ischemic period (20 min—four time points) and recovery period (8 min—one time point). The baseline, pre-ischemic and recovery period differences were statistically tested for intergroup differences using Student's *t*-test. The ischemic and reperfusion periods were statistically tested using repeated measures analysis of variance, followed by a Student's *t*-test with Bonferroni correction for intergroup differences. Statistical analysis was performed using SPSS (version 8.0.0, SPSS, USA). Differences were considered to be statistically significant at *P*-values lower than 0.05.

3. Results

3.1. Hemodynamic readings

Fig. 1 shows a graphical representation of the coronary blood flow before and after ischemia (baseline, pre-ischemic, post-ischemic and recovery periods) for 4 μmol l^{-1} glibenclamide or vehicle. As shown in Table 1, there are no differences in baseline aorta flow or between groups. The 10-min pre-ischemic infusion of 4 μmol l^{-1} glibenclamide decreased the coronary blood flow to 59.5±7.0% of control vs. 94.3±1.3% for vehicle ($P=0.008$). During the post-ischemic period, the glibenclamide-induced reduction in coronary blood flow is even more pronounced (45.6±5.4% vs. 104.4±5.9% for vehicle, $P<0.0001$) (Fig. 2A). During the recovery period, the coronary blood flow increases seen with vehicle (due to the higher metabolic demand) are significantly lower in the glibenclamide group (68.9±7.8% vs. 137.4±10.2%, $P=0.0007$) (Fig. 2A).

Table 1
Hemodynamic effects of vehicle and glibenclamide at baseline and recovery periods

	Vehicle (n=5)	4 μmol l^{-1} glibenclamide (n=5)
Aortic blood flow (ml min^{-1})		
Baseline	38.6±1.3	40.8±2.3
Recovery	27.6±1.6	40.2±2.4
Coronary blood flow (ml min^{-1})		
Baseline	7.3±1.3	10.1±1.0
Recovery	9.8±1.4	6.9±0.9

Absolute aortic blood flow and absolute coronary blood flow are shown. Data are presented as mean±standard error of the mean (S.E.M.).

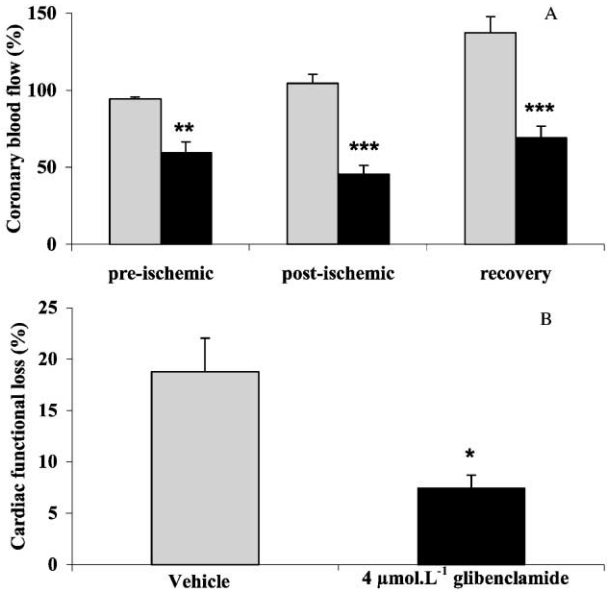

Fig. 2. Hemodynamic changes induced by vehicle (grey bars) and 4 μmol l^{-1} glibenclamide (black bars). Effects on coronary blood flow in the pre-ischemic, post-ischemic and recovery period relative to baseline values are shown in panel A. Effects on post-ischemic cardiac functional loss are shown in panel B. Cardiac functional loss is expressed as post-ischemic cardiac function (aorta flow + coronary blood flow) relative to pre-ischemic cardiac function. Data are represented as mean±standard error of the mean. *$P<0.05$, **$P<0.01$ and ***$P<0.001$ compared with vehicle.

The 12-min global ischemic insult resulted in a significant amount of cardiac functional loss (18.8±3.3%), which was significantly reduced by glibenclamide (7.4±1.3%, $P=0.019$) (Fig. 2B).

3.2. Metabolic readings

Fig. 3 shows representative spectra of one experiment in presence of 4 μmol l^{-1} glibenclamide. During the baseline period, no significant differences in relative Pi, phosphocreatine and ATP levels were observed between vehicle and 4 μmol l^{-1} glibenclamide (Fig. 4A,B and C). The calculated ATP:phosphocreatine ratios were also comparable (vehicle: 0.66±0.07 vs. 0.59±0.04 for glibenclamide). The Pi level was significantly higher during pre-ischemic period in the vehicle group compared with the glibenclamide group (0.97±0.27 vs. 0.31±0.10, $P=0.05$) (Fig. 4A), while for phosphocreatine and ATP, there were no differences during

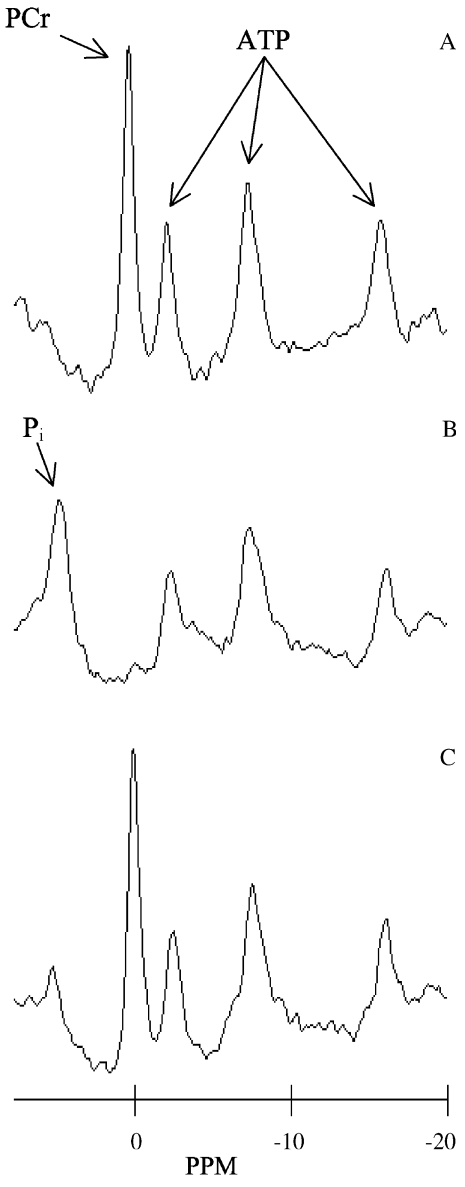

Fig. 3. Representative ^{31}P-magnetic resonance spectra of (A) baseline, (B) ischemia and (C) recovery periods after 4 μmol l^{-1} glibenclamide treatment. Peaks of Pi, phosphocreatine (PCr) and ATP are shown.

the pre-ischemic period (Fig. 4B and C). The calculated intracellular pH was not significantly different between groups during either baseline or pre-ischemic periods (Fig. 4D).

During the ischemic period, the rise in relative Pi levels and the drop in relative phosphocreatine and ATP levels were not significantly different between vehicle and glibenclamide (Fig. 4A and B). The ischemia-induced drop in intracellular pH was significantly attenuated in the glibenclamide group compared with the vehicle group ($P=0.03$), with a final pH after 12 min ischemia of 6.75 ± 0.05 vs. 6.43 ± 0.06 ($P=0.03$) (Fig. 4D).

During the post-ischemic and recovery periods, the relative phosphocreatine and ATP levels were similar in

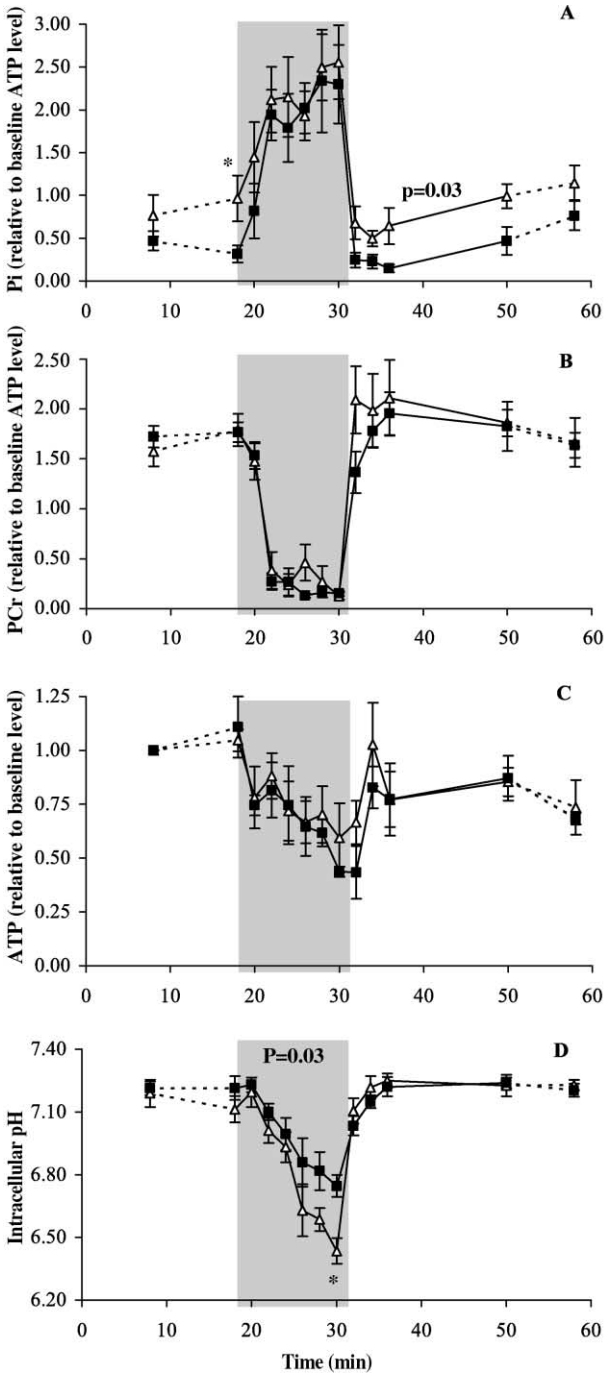

Fig. 4. Metabolic readings throughout the whole experimental protocol (baseline, pre-ischemic, post-ischemic and recovery periods) of vehicle (△) and 4 μmol l^{-1} glibenclamide (■). In (A), the Pi levels are shown relative to the baseline ATP level. In (B), the phosphocreatine levels are shown relative to the baseline ATP level. In (C), the relative ATP levels (β-ATP peak) are shown as a ratio to baseline level. In (D), the calculated intracellular pH is shown. Results are presented as mean±standard error of the mean (S.E.M.). The shaded areas represent the 12-min global ischemic interval and the dotted lines discriminate between baseline/pre-ischemic and post-ischemic/recovery periods, respectively. *$P<0.03$ vs. vehicle.

both groups (Fig. 4B and C). Relative Pi levels were significantly higher post-ischemically in the vehicle group compared with the glibenclamide group ($P=0.01$), although during the recovery period, the Pi levels were similar again (Fig. 4A). No significantly different intracellular pH levels were observed during the post-ischemic and recovery periods between in either group (Fig. 4D).

4. Discussion

Glibenclamide reduces coronary blood flow, which may be completely attributed to vascular K_{ATP} channel blockade. Furthermore, glibenclamide improves post-ischemic cardiac function preserving intracellular pH but not intracellular ATP levels during ischemia. This suggests that the beneficial response to glibenclamide is probably not the result of myocardial K_{ATP} channel blockade, but may be explained by inhibition of glycolysis.

4.1. Model considerations and clinical relevance

Effects of sulfonylurea derivatives on post-ischemic cardiac function are difficult to determine in a clinically relevant fashion. It is difficult to study these mechanisms in humans, so animal research facilitate exploration of the actions of glibenclamide. In the majority of these studies, isolated, perfused animal hearts were used. These models do have some avoidable limitations.

First of all, most isolated hearts are perfused with crystalloid buffer, to provide oxygen and metabolic supplies to the heart. However, it has been shown previously that perfusing hearts with crystalloid buffer, without the presence of an oxygen carrier (e.g. hemoglobin), results in higher coronary blood flow levels (near maximal vasodilation); in fact, hearts are at the brink of being hypoxic during normal baseline situations (Olders et al., 1990b). This is illustrated by the three-fold decline in coronary blood flow levels after switching from crystalloid perfused buffer to erythrocyte-enriched crystalloid buffer in our setup (Olders et al., 1990b). Therefore, interpretation of ischemia-induced and vasoactive effects is much more difficult because baseline registrations are not completely free of ischemia.

A second point of importance is the lack of protein in crystalloid perfused hearts. The presence of protein is needed to avoid edema (Podesser et al., 1999), and also reflects the in vivo condition of patients treated by sulfonylurea derivatives more precisely than protein free perfusion fluid. Sulfonylureas are highly protein bound drugs (>98%) and the dynamic equilibrium between the bound and the active non-bound fraction of the drugs is also applying to our model. Also, the isolated heart model mostly used is the so-called Langendorff heart setup instead of the preferable working heart setup. Because the working heart setup actually performs external work, this model is one step further in the direction of clinical relevance than the Langendorff heart setup. In addition it has been shown that the working heart is more sensitive to ischemic damage than the Langendorff heart setup (Galinanes and Hearse, 1990; Podesser et al., 1999; Smolens et al., 1995).

Due to the limited clinical relevance, conclusions from studies in the Langendorff model are restricted. Therefore, we performed the present study using an isolated working rat heart setup, which was perfused with a crystalloid buffer, enriched with erythrocytes and protein to improve the clinical relevance of observations on coronary blood flow, functional recovery and metabolic effects post-ischemically.

4.2. Hemodynamic effects

4.2.1. Coronary blood flow

The observed decrease in coronary blood flow after glibenclamide treatment during normoxia is consistent with previous observations in open-chest dogs (Imamura et al., 1992; Samaha et al., 1992), and in isolated rat hearts (Legtenberg et al., 2001). These observations suggest that the ion flux across K_{ATP} channels in vascular smooth muscle cells of coronary arteries contributes to baseline coronary vascular tone. Interestingly, the vasoconstrictor effect of the drug seemed to be more pronounced in the post-ischemic period. This agrees with the view that the open-state probability of K_{ATP} channels is higher during and after ischemia than under resting conditions (Coetzee, 1992; Noma, 1983).

Interpretation of the clinical relevance of the vasoconstrictor response to glibenclamide is difficult, because in the present study healthy rats were used instead of diabetic rats. Since diabetes impairs regional blood flow and many of these patients have coronary artery disease, the importance of the vasoconstrictor effect of the sulfonylurea derivatives may actually be underestimated in the present study. Furthermore, in this study, we used a concentration that is slightly higher than therapeutic, but we previously showed that with therapeutic concentrations of glibenclamide a vasoconstrictor response is also present, although to a lesser extent (Legtenberg et al., 2001).

4.2.2. Cardiac function

Glibenclamide reduced the ischemia-induced loss of cardiac function in the present study. This was consistent with previous observations in isolated hearts (Ali et al., 1993; Docherty et al., 1997; Legtenberg et al., 2001; Tosaki and Hellegouarch, 1994). Consistent with this, intracellular Pi levels were significantly lower post-ischemically due to glibenclamide treatment, also indicating an improved energetic status post-ischemically. On the other hand, it has been found that glibenclamide increases the infarct size developing after ischemia and it can abolish the beneficial effect on infarct size due to ischemic preconditioning (Gross and Auchampach, 1992; Munch-Ellingsen et al., 1996; Qian et

al., 1996; Rohmann et al., 1994; Thornton et al., 1993). The detrimental effect of glibenclamide on infarct size can be explained by the depolarization of the myocardial membrane. This results in an increase of Ca^{2+} influx, thereby prolonging the ischemia-induced shortening of the action potential and increasing energy utilization. In a situation of infarct development, this increased energy utilization is damaging to the heart.

In the present study, intracellular pH was preserved by glibenclamide during ischemia, which is consistent with previous observations (Docherty et al., 1997). In the study of Docherty et al., ATP levels were also depleted at a faster rate during ischemia by glibenclamide, and they concluded that inhibition of glycolysis during ischemia contributes to the protective mechanism of glibenclamide in their model, which was consistent with a previous observation (Schaffer et al., 1985). In addition, it has been shown that the involvement of lactate may be important in the observed reduction in functional loss in ventricular myocytes (Lederer and Nichols, 1989). In the present study, ATP levels were not depleted at a significantly faster rate, but ATP levels were also not preserved during ischemia. Therefore, the present result may indicate that inhibition of glycolysis contributes to the observed protective mechanism of glibenclamide.

Glibenclamide has been shown to uncouple mitochondrial oxidative phosphorylation with a K_d of 4 μmol l^{-1} (Szewczyk et al., 1997). Furthermore, it has been shown that uncoupling of mitochondrial oxidative phosphorylation with the classical uncoupling agent dinitrophenol also protects post-ischemic cardiac function, which supports the concept that stressful stimuli to the mitochondrion may result in cardioprotection, in the same way as ischemic preconditioning (Minners et al., 2000). In addition, it is believed that the protective effect of opening mitochondrial K_{ATP} channels is potentiated by uncoupling of the oxidative phosphorylation (Garlid, 1996; Garlid et al., 1997; Holmuhamedov et al., 1998; Miyamae et al., 1996). When uncoupling of mitochondrial oxidative phosphorylation is an important contributor to the preservation of post-ischemic cardiac function in the present study, phosphocreatine or ATP levels should decline at a much faster rate during ischemia and recovery, which was not observed in this study. This therefore does not support a significant role for uncoupling of mitochondrial oxidative phosphorylation in our model.

It has been proposed that glibenclamide preserves ATP levels during ischemia (Tosaki and Hellegouarch, 1994). By preserving intracellular ATP levels, hearts would be less energy depleted after ischemia, and therefore they might recover better. However, in the present study, glibenclamide did not preserve intracellular ATP levels during ischemia, indicating that this does not play a role in the cardioprotection observed.

Interestingly, the vasoconstrictor response to glibenclamide did not impair post-ischemic cardiac function. Apparently, the resulting reduced coronary blood flow is high enough to support the improved myocardial function after ischemia. Our data suggest that the vasoconstrictive effect of glibenclamide is probably due to direct blockade of vascular smooth muscle K_{ATP} channels, while the improvement of post-ischemic function is probably due to other mechanisms. Therefore, a possible detrimental effect of the reduction in coronary blood flow may be masked by other mechanisms triggered by glibenclamide.

4.3. Future considerations

Future experiments are needed to know whether the effects by glibenclamide reported in this study are specific to glibenclamide only. It would be very interesting to know what the effects of glimepiride, a newer sulfonylurea derivative, are on intracellular ATP levels. In a previous study of our department, we showed that glimepiride also improved post-ischemic cardiac function as does glibenclamide (Legtenberg et al., 2001). Furthermore, is it of interest to compare the present results with data from 5-hydroxydecanoate, a specific mitochondrial K_{ATP} channel blocker. Differences would be expected because the effects of glibenclamide on intracellular pH in this study are likely to occur not through direct K_{ATP} channel blockade, while 5-hydroxydecanoate directly inhibits mitochondrial K_{ATP} channels.

4.4. Conclusion

Glibenclamide reduces coronary blood flow both during normoxic and post-ischemic tissue perfusion, indicating that the coronary vasomotor action of glibenclamide is mediated by blockade of vascular K_{ATP} channels. Furthermore, glibenclamide improves post-ischemic cardiac function preserving intracellular pH but not intracellular ATP levels during ischemia. This suggests that the beneficial response to glibenclamide is probably not the result of myocardial K_{ATP} channel blockade, but may be explained, at least in part, by inhibition of glycolysis.

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References

Ali, K., Morimoto, M., Fukaya, Y., Furukawa, Y., 1993. Improvement of cardiac function impaired by repeated ischemic arrests in isolated rat hearts. Ann. Thorac. Surg. 55, 902–907.

Coetzee, W.A., 1992. ATP-sensitive potassium channels and myocardial ischemia: why do they open? [editorial] . Cardiovasc. Drugs Ther. 6, 201–208.

Docherty, J.C., Gunter, H.E., Kuzio, B., Shoemaker, L., Yang, L., Deslauriers, R., 1997. Effects of cromakalim and glibenclamide on myocardial high energy phosphates and intracellular pH during ischemia–reperfusion: ^{31}P NMR studies. J. Mol. Cell. Cardiol. 29, 1665–1673.

Galinanes, M., Hearse, D.J., 1990. Assessment of ischemic injury and protective interventions: the Langendorff versus the working rat heart preparation. Can. J. Cardiol. 6, 83–91.

Garlid, K.D., 1996. Cation transport in mitochondria—the potassium cycle. Biochim. Biophys. Acta 1275, 123–126.

Garlid, K.D., Paucek, P., Yarov-Yarovoy, V., Murray, H.N., Darbenzio, R.B., D'Alonzo, A.J., Lodge, N.J., Smith, M.A., Grover, G.J., 1997. Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K+ channels. Possible mechanism of cardioprotection. Circ. Res. 81, 1072–1082.

Gerich, J.E., 1989. Oral hypoglycemic agents [published erratum appears in N. Engl. J. Med. 1990 Jan 4;322(1):71]. N. Engl. J. Med. 321, 1231–1245.

Groop, L.C., 1992. Sulfonylureas in NIDDM. Diabetes Care 15, 737–754.

Gross, G.J., Auchampach, J.A., 1992. Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs. Circ. Res. 70, 223–233.

Holmuhamedov, E.L., Jovanovic, S., Dzeja, P.P., Jovanovic, A., Terzic, A., 1998. Mitochondrial ATP-sensitive K+ channels modulate cardiac mitochondrial function. Am. J. Physiol. 275, H1567–H1576.

Houston, R.J., Heerschap, A., Skotnicki, S.H., Verheugt, F.W., Oeseburg, B., 1997. ATP content measured by ^{31}P NMR correlates with post-ischaemic recovery of external work output in an isolated rat heart perfused with erythrocyte suspension. J. Mol. Cell. Cardiol. 29, 1763–1766.

Houston, R.J., Legtenberg, R.J., Heerschap, A., Oeseburg, B., 1998. Improved air temperature control system for isolated organ NMR experiments. MAGMA 6, 195.

Imamura, Y., Tomoike, H., Narishige, T., Takahashi, T., Kasuya, H., Takeshita, A., 1992. Glibenclamide decreases basal coronary blood flow in anesthetized dogs. Am. J. Physiol. 263, H399–H404.

Jacobus, W.E., Pores, I.H., Taylor, G.J., Nunnally, R.L., Hollis, D.P., Weisfeldt, M.L., 1978. Tight coupling of intracellular pH and ventricular performance. J. Mol. Cell. Cardiol. 10, 39.

Lederer, W.J., Nichols, C.G., 1989. Nucleotide modulation of the activity of rat heart ATP-sensitive K+ channels in isolated membrane patches. J. Physiol. (London) 419, 193–211.

Legtenberg, R.J., Houston, R.J., Oeseburg, B., Smits, P., 2001. Effects of sulfonylurea derivatives on ischemia-induced loss of function in the isolated rat heart. Eur. J. Pharmacol. 419, 85–92.

Minners, J., van den Bos, E.J., Yellon, D.M., Schwalb, H., Opie, L.H., Sack, M.N., 2000. Dinitrophenol, cyclosporin A, and trimetazidine modulate preconditioning in the isolated rat heart: support for a mitochondrial role in cardioprotection. Cardiovasc. Res. 47, 68–73.

Miyamae, M., Camacho, S.A., Weiner, M.W., Figueredo, V.M., 1996. Attenuation of postischemic reperfusion injury is related to prevention of [Ca2+]m overload in rat hearts. Am. J. Physiol. 271, H2145–H2153.

Munch-Ellingsen, J., Bugge, E., Ytrehus, K., 1996. Blockade of the KATP-channel by glibenclamide aggravates ischemic injury, and counteracts ischemic preconditioning. Basic Res. Cardiol. 91, 382–388.

Nichols, C.G., Ripoll, C., Lederer, W.J., 1991. ATP-sensitive potassium channel modulation of the guinea pig ventricular action potential and contraction. Circ. Res. 68, 280–287.

Noma, A., 1983. ATP-regulated K+ channels in cardiac muscle. Nature 305, 147–148.

Olders, J., Boumans, T., Evers, J., Turek, Z., 1990a. An experimental set-up for the blood perfused working isolated rat heart. Adv. Exp. Med. Biol. 277, 151–160.

Olders, J., Turek, Z., Evers, J., Hoofd, L., Oeseburg, B., Kreuzer, F., 1990b. Comparison of Tyrode and blood perfused working isolated rat hearts. Adv. Exp. Med. Biol. 277, 403–413.

Podesser, B.K., Hallstrom, S., Schima, H., Huber, L., Weisser, J., Kroner, A., Furst, W., Wolner, E., 1999. The erythrocyte-perfused "working heart" model: hemodynamic and metabolic performance in comparison to crystalloid perfused hearts. J. Pharmacol. Toxicol. Methods 41, 9–15.

Qian, Y.Z., Levasseur, J.E., Yoshida, K., Kukreja, R.C., 1996. KATP channels in rat heart: blockade of ischemic and acetylcholine-mediated preconditioning by glibenclamide. Am. J. Physiol. 271, H23–H28.

Rohmann, S., Weygandt, H., Schelling, P., Kie Soei, L., Verdouw, P.D., Lues, I., 1994. Involvement of ATP-sensitive potassium channels in preconditioning protection. Basic Res. Cardiol. 89, 563–576.

Russel, F.G., van Uum, S., Tan, Y., Smits, P., 1998. Solid-phase extraction of 18beta-glycyrrhetinic acid from plasma and subsequent analysis by high-performance liquid chromatography. J. Chromatogr., B: Biomed. Sci. Appl. 710, 223–226.

Samaha, F.F., Heineman, F.W., Ince, C., Fleming, J., Balaban, R.S., 1992. ATP-sensitive potassium channel is essential to maintain basal coronary vascular tone in vivo. Am. J. Physiol. 262, C1220–C1227.

Schaffer, S.W., Tan, B.H., Mozaffari, M.S., 1985. Effect of glyburide on myocardial metabolism and function. Am. J. Med. 79, 48–52.

Smolens, I.A., Follette, D.M., Berkoff, H.A., Castellanos, L.M., Segel, L.D., 1995. Incomplete recovery of working heart function after twenty-four-hour preservation with a modified University of Wisconsin solution. J. Heart Lung Transplant 14, 906–915.

Szewczyk, A., Czyz, A., Nalecz, M.J., 1997. ATP-regulated potassium channel blocker, glibenclamide, uncouples mitochondria. Pol. J. Pharmacol. 49, 49–52.

Thornton, J.D., Thornton, C.S., Sterling, D.L., Downey, J.M., 1993. Blockade of ATP-sensitive potassium channels increases infarct size but does not prevent preconditioning in rabbit hearts. Circ. Res. 72, 44–49.

Tosaki, A., Hellegouarch, A., 1994. Adenosine triphosphate-sensitive potassium channel blocking agent ameliorates, but the opening agent aggravates, ischemia/reperfusion-induced injury. Heart function studies in nonfibrillating isolated hearts. J. Am. Coll. Cardiol. 23, 487–496.

Van den Boogaart, A., Howe, F.A., Rodrigues, L.M., Stubbs, M., Griffiths, J.R., 1995. In vivo ^{31}P MRS: absolute concentrations, signal-to-noise and prior knowledge. NMR Biomed. 8, 87–93.

Van der Veen, J.W., De Beer, R., Luyten, P.R., van Ormondt, D., 1988. Accurate quantification of in vivo ^{31}P NMR signals using the variable projection method and prior knowledge. Magn. Reson. Med. 6, 92–98.