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European Journal of Pharmacology 419 (2001) 85–92

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Effects of sulfonylurea derivatives on ischemia-induced loss of function in the isolated rat heart

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Received 11 January 2001; received in revised form 3 April 2001; accepted 6 April 2001

Abstract

This study determined whether sulfonylurea derivatives affect cardiac function prior to and after a mild ischemic incident (stunning). This was investigated using an isolated, erythrocyte-perfused, working rat heart model. In total, 11 groups were studied: five increasing (clinically relevant) concentrations of the classical *glibenclamide* (range 0.005–4 μmol l^{-1}), five increasing concentrations of the newly developed *glimepiride* (range 0.005–0.8 μmol l^{-1}), and one control group. Pre-ischemically, glibenclamide and glimepiride reduced coronary blood flow concentration dependently to $55.2 \pm 4.5\%$ and $58.5 \pm 5.5\%$, respectively ($P < 0.001$). Twenty minutes after a 12-min ischemic incident, these reductions of flow were even more pronounced (to $38.3 \pm 6.7\%$ and $45.8 \pm 5.8\%$, $P < 0.001$). This shows that both sulfonylureas reduce coronary blood flow at concentrations slightly higher than therapeutic ones. In the control group, the ischemic incident significantly lowered cardiac function by $22.2 \pm 2.9\%$. In the therapeutic range, glimepiride, but not glibenclamide, significantly reduced this ischemia-induced cardiac functional loss to $4.9 \pm 1.2\%$ ($P < 0.01$). Therefore, we suggest that both sulfonylureas and in particular glimepiride can be used safely in patients with type 2 diabetes mellitus, as long as the coronary vascular system is not compromised. Because of the obvious vasocontrictor response to sulfonylurea derivatives, these drugs must be used with caution in patients with a reduced coronary reserve.

Keywords: Diabetes type 2; K_{ATP} channel; Cardiovascular; Heart; Ventricular

1. Introduction

Sulfonylurea derivatives are still the cornerstone of the treatment of type 2 diabetes mellitus (Groop, 1992; Gerich, 1989). These drugs exert their blood glucose lowering effect by stimulating insulin secretion in the pancreatic β-cell (Groop, 1992; Gerich, 1989). Although this mechanism of action has been known for decades, the molecular target for sulfonylurea derivatives was discovered only recently (Ashcroft, 1996). Sulfonylurea derivatives are able to bind to a specific sulfonylurea receptor (SUR_1), which is a principal component of the so-called ATP-sensitive K^+ channel (K_{ATP} channel). By blocking K^+ efflux, sulfonylurea derivatives depolarize the plasma membrane, which triggers the opening of voltage-dependent Ca^{2+} channels. The subsequent influx of Ca^{2+} ultimately results in insulin release (Ashcroft, 1996; Noma, 1989).

Functional K_{ATP} channels have also been identified in the cardiovascular system (Noma, 1989). The sulfonylurea receptors in myocardial and in vascular smooth muscle cells are slightly different from the pancreatic SUR_1, and have been characterized as SUR_{2a} and SUR_{2b}, respectively (Chutkow et al., 1996; Inagaki et al., 1996; Isomoto et al. 1995). Although classical drugs such as tolbutamide and glibenclamide have a much higher affinity at SUR_1 than at SUR_{2a} and SUR_{2b} (Yokoshiki et al., 1998), it is still unknown whether therapeutic concentrations of these drugs affect the function of the cardiovascular system. Theoretically, blockade of the cardiovascular K_{ATP} channel may interfere with endogenous cardioprotective mechanisms, especially during states of ischemia, when reduced levels of intracellular ATP trigger the opening of K_{ATP} channels (Coetzee, 1992; Nichols et al., 1991; Noma, 1989). High concentrations of sulfonylurea derivatives have been shown to block several beneficial or detrimental

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0014-2999/01/$ - see front matter
PII: S0014-2999(01)00974-8

ischemia-induced phenomena, including coronary vasodilation (Aversano et al., 1991; Daut et al., 1990), arrhythmias (El-Reyani et al., 1999; Tosaki and Hellegouarch, 1994; Billman et al., 1993), ischemic preconditioning (Klepzig et al., 1999; Gross and Auchampach, 1992), loss of cardiac function (Docherty et al., 1997; Tosaki and Hellegouarch, 1994; Ali et al., 1993; Mitani et al., 1991) and myocardial stunning (Auchampach et al., 1992).

Many different models and protocols have been used to study the effects of sulfonylureas on cardiac hemodynamics. The primary model used to study hemodynamics before and after ischemia is an isolated heart model. In general, these isolated hearts are perfused with crystalloid oxygenated buffers. Due to the lower oxygen capacity of these buffers (lack of hemoglobin), the coronary blood flow in these models is much higher than in the in vivo situation, in fact there is near maximal vasodilation. Therefore, vasodilator responses and the effects of sulfonylureas on coronary blood flow are difficult to determine in these models (Podesser et al., 1999; Olders et al., 1990b). Furthermore, most groups have used a Langendorff perfused heart (Langendorff, 1895) instead of a working heart set-up (Neely et al., 1967). This can also be a source of difficulty in interpreting the clinical relevance of results. Another important point in interpreting results is to consider whether infarct size or functional recovery is measured. Also the differences between reversible ischemic damage (e.g. stunning) and irreversible ischemic damage (infarct) have to be taken into account.

Therefore, we used an isolated working rat heart model. To the modified Krebs–Henseleit buffer we added bovine erythrocytes and albumin. The use of erythrocytes makes the coronary blood flow more like that in vivo, so results are better interpretable. The use of albumin gives a better view of the pharmacological effects of sulfonylureas in rat hearts as drug–protein interaction occurs and thus extrapolation to the in vivo situation in patients treated with these drugs is improved.

The objective of the present study was to address the myocardial safety of therapeutic concentrations of sulfonylurea derivatives in this novel and useful model of myocardial ischemia. We investigated two sulfonylurea derivatives, *glibenclamide*, because this is the most widely used classical representative of this class of drugs, and *glimepiride*, because this sulfonylurea derivative has been shown to be more selective for the SUR_1 receptor (Bijlstra et al., 1996; Geisen et al., 1996). Finally, drugs were used over a large concentration range, including therapeutic concentrations.

2. Methods

2.1. Reagent and experimental groups

Glibenclamide was purchased from Sigma-Aldrich Chemie, Zwijndrecht, The Netherlands. Glimepiride was kindly provided by Hoechst Marion Roussel, The Netherlands. In total, 50 animals were used in 11 experimental groups: vehicle ($n = 8$); and increasing concentrations of glibenclamide: 0.005, 0.05, 0.25, 1 and 4 μmol l^{-1} ($n = 3$ to 5) and glimepiride 0.005, 0.05, 0.15, 0.25 and 0.8 μmol l^{-1} ($n = 3$ to 5). 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 concentrations were free non-protein bound concentrations (measured by high-performance liquid chromatography (HPLC) analysis following equilibrium analysis, see Russel et al., 1998). Both sulfonylurea derivatives were highly bound to protein (97.5%), and therefore we used the free unbound concentrations. For example, a "free" concentration of 4 μmol l^{-1} glibenclamide was achieved by applying a "total" concentration of 160 μmol l^{-1} glibenclamide. For glimepiride, 0.8 μmol l^{-1} was the highest achievable unbound concentration without crystallization.

2.2. Animal model

A more detailed description of the perfusion set-up has already been published elsewhere (Olders et al., 1990a). Briefly, a male Wistar rat (400 g nominal weight; 4–6 months old) was anesthetized with diethyl ether, and the heart was excised by 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, pacing-wires were attached and the hearts were paced at around 360 bpm (delay time = 1 ms, voltage = 5 V). After checking for leaks, the system was switched to the working configuration with an 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 modified Krebs–Henseleit buffer solution was as follows: NaCl 118, $CaCl_2$ 3.0, KCl 4.7, $NaHCO_3$ 25, $MgSO_4$ 1.2, KH_2PO_4 1.2, NaEDTA 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 addition of erythrocytes to give a hematocrit of 0.25. The free Ca^{2+} concentration in this suspension was 1.6 mmol l^{-1} as determined by blood gas analysis. The temperature of the heart and perfusate was maintained throughout the experiment at normothermia of a rat (38°C).

2.3. Experimental protocol and statistical analysis

After an equilibrium period of approximately 15 min, 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 aortic 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). 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 data presented in Section 3 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. The flow debt repayment post-ischemically is expressed as the total summed coronary blood flow during the 20-min post-ischemic period relative to the coronary blood flow during the last minute (8th min) of the baseline period (if coronary blood flow had remained a constant 100% throughout the 20-min post-ischemic period, this total would be 2000).

The results are presented as means ± standard error of the mean (S.E.M.). Differences in coronary blood flow and cardiac functional loss were statistically tested using one-way analysis of variance (ANOVA) followed by a Bonferroni comparison test. The Bonferroni comparison test was only performed when the one-way ANOVA was significant. One-site competition curve fitting was done to determine the concentration–response relationships of the coronary blood flow effects. Differences in EC_{50} were tested using the Welch corrected *t*-test. Statistical analysis was performed using Instat (version 3.00, Graphpad Software San Diego, USA). Curve fitting was performed using Prism (version 2, Graphpad Software). For the curve fitting, an r^2-value higher than 0.95 was considered reliable. Differences were considered to be statistically significant at P values lower than 0.05.

3. Results

Fig. 1 shows a graphical representation of the baseline coronary blood flow, and of the effects of vehicle and of both sulfonylurea derivatives (4 μmol l^{-1} glibenclamide and 0.8 μmol l^{-1} glimepiride) on coronary blood flow before and after ischemia (baseline, pre-ischemic, post-ischemic and recovery periods).

The absolute data on coronary blood flow as well as the data on aortic blood flow after administration of vehicle or drugs during the baseline and recovery periods are summarized in Table 1. Both glibenclamide and glimepiride

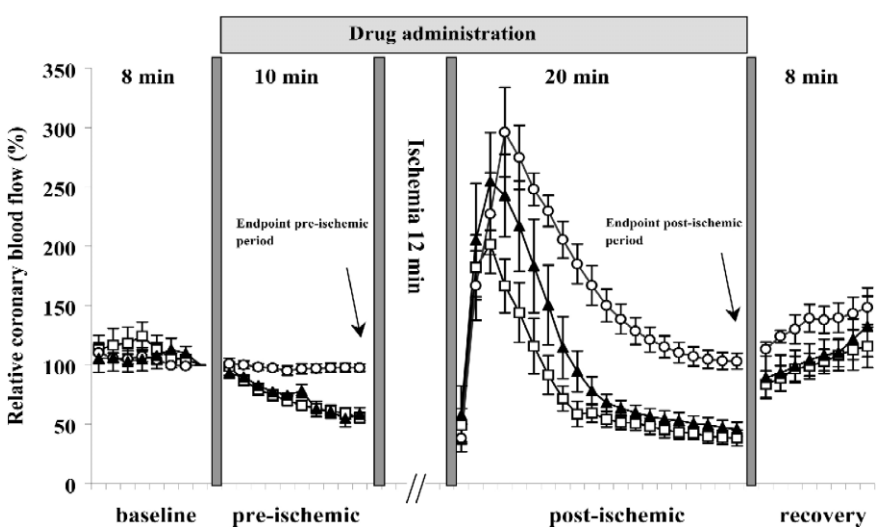

Fig. 1. Coronary blood flow tracings throughout the whole experimental protocol (baseline, pre-ischemic, post-ischemic and recovery periods) for vehicle (○), 4 μmol l^{-1} glibenclamide (□), and 0.8 μmol l^{-1} glimepiride (▲).

Table 1
Hemodynamic effects of sulfonylurea derivatives at baseline and recovery periods
Effects of vehicle, glibenclamide (Glib) and glimepiride (Glim) on cardiac hemodynamics at baseline and recovery periods. Absolute aortic blood flow and absolute coronary blood flow are shown. Data are presented as means ± standard error of the mean (S.E.M.).

	Aortic blood flow (ml min^{-1} ± S.E.M.)		Coronary blood flow (ml min^{-1} ± S.E.M.)	
	Baseline	Recovery	Baseline	Recovery
Vehicle ($n = 8$)	58.3 ± 3.1	42.0 ± 2.7	9.1 ± 0.4	10.3 ± 0.7
0.005 μmol l^{-1} Glib ($n = 5$)	60.8 ± 3.9	44.6 ± 5.9	10.5 ± 1.9	12.8 ± 2.0
0.05 μmol l^{-1} Glib ($n = 5$)	58.8 ± 3.4	46.8 ± 5.9	10.4 ± 1.9	11.5 ± 1.8
0.25 μmol l^{-1} Glib ($n = 3$)	60.3 ± 12.2	47.0 ± 11.0	7.8 ± 0.8	9.1 ± 1.3
1 μmol l^{-1} Glib ($n = 3$)	67.7 ± 4.3	62.7 ± 6.8	8.5 ± 0.3	7.2 ± 1.2
4 μmol l^{-1} Glib ($n = 5$)	62.8 ± 4.0	61.4 ± 4.1	8.7 ± 0.8	7.1 ± 0.8
0.005 μmol l^{-1} Glim ($n = 5$)	69.2 ± 2.0	62.4 ± 3.4	9.0 ± 1.3	11.9 ± 1.4
0.05 μmol l^{-1} Glim ($n = 5$)	66.2 ± 2.0	62.2 ± 2.6	8.9 ± 0.6	9.3 ± 0.5
0.15 μmol l^{-1} Glim ($n = 3$)	69.7 ± 1.2	57.3 ± 3.7	7.6 ± 0.4	9.3 ± 0.4
0.25 μmol l^{-1} Glim ($n = 3$)	68.7 ± 1.9	51.3 ± 51.3	9.2 ± 0.6	8.6 ± 0.7
0.8 μmol l^{-1} Glim ($n = 5$)	72.4 ± 2.6	60.0 ± 3.7	9.7 ± 1.6	8.4 ± 1.7

reduced coronary blood flow concentration dependently during the pre-ischemic period (Table 2). This concentration-dependent vasoconstrictor effect of glibenclamide and glimepiride was even more pronounced in the post-ischemic period as compared with the pre-ischemic period (glibenclamide: E_{max} post-ischemically 38.3 ± 6.7% vs. pre-ischemically 55.2 ± 4.5%—$P < 0.01$; glimepiride: E_{max} post-ischemically 45.8 ± 5.8% vs. pre-ischemically 58.5 ± 5.5%—$P < 0.05$).

One-site competition curve-fitted concentration–response relationships are shown in Fig. 2 for both drugs during the pre- vs. the post-ischemic period. For all four curves, the r^2 values were above 0.95, indicating reliable curve fits. Pre-ischemically, the calculated log EC_{50} values were not significantly different between both sulfonylurea derivatives: −6.6 ± 0.3 (0.28 μmol l^{-1}) and −6.8 ± 0.5 (0.17 μmol l^{-1}) for glibenclamide and glimepiride, respectively ($P = 0.70$). Post-ischemically, a small tendency towards lower EC_{50} values was observed: −6.9 ± 0.1 (0.13

Table 2
Hemodynamic effects of sulfonylurea derivatives in pre- and post-ischemic periods
Effects of vehicle, glibenclamide (Glib) and glimepiride (Glim) on cardiac hemodynamics at the end of pre-ischemic and post-ischemic periods. Coronary blood flow data are shown normalized to baseline level (8th min of baseline period). Data are presented as means ± standard error of the mean (S.E.M.).

	Normalized coronary blood flow (% ± S.E.M.)	
	Pre-ischemic	Post-ischemic
Vehicle ($n = 8$)	97.4 ± 3.1	103.0 ± 6.6
0.005 μmol l^{-1} Glib ($n = 5$)	87.9 ± 5.9	100.5 ± 8.2
0.05 μmol l^{-1} Glib ($n = 5$)	78.7 ± 3.1[a]	84.3 ± 6.9
0.25 μmol l^{-1} Glib ($n = 3$)	74.5 ± 2.2[a]	56.7 ± 10.1[b]
1 μmol l^{-1} Glib ($n = 3$)	63.0 ± 7.4[c]	36.4 ± 2.2[c]
4 μmol l^{-1} Glib ($n = 5$)	55.2 ± 4.5[c]	38.3 ± 6.7[c]
0.005 μmol l^{-1} Glim ($n = 5$)	97.1 ± 8.5	101.0 ± 9.3
0.05 μmol l^{-1} Glim ($n = 5$)	86.4 ± 4.5	74.3 ± 6.7
0.15 μmol l^{-1} Glim ($n = 3$)	70.0 ± 7.8[b]	58.5 ± 13.0[b]
0.25 μmol l^{-1} Glim ($n = 3$)	80.8 ± 2.6	61.0 ± 4.3[b]
0.8 μmol l^{-1} Glim ($n = 5$)	58.5 ± 5.5[c]	45.8 ± 5.8[c]

[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.

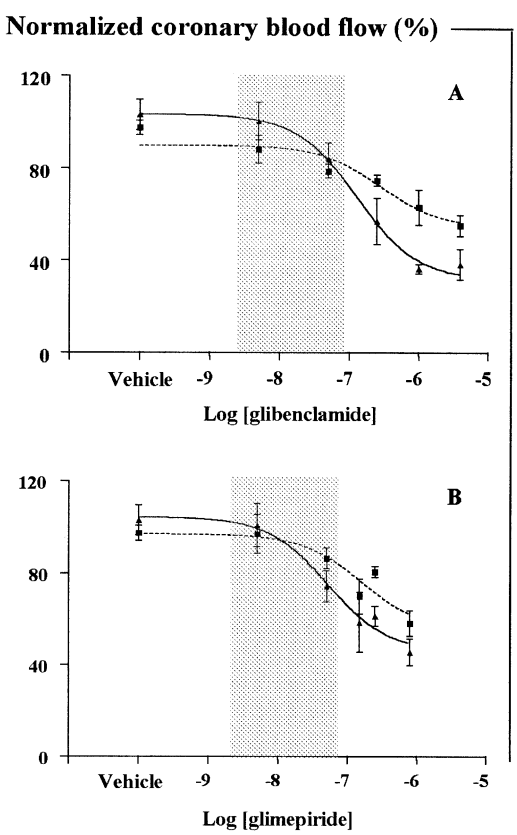

Fig. 2. One-site competition curve fitting for normalized coronary blood flow effects induced by pre/post-ischemic glibenclamide (A) and glimepiride (B) treatment. The dotted lines and squares represent the pre-ischemic curves, while the solid lines and triangles represent the post-ischemic curves. The shaded areas represent the therapeutic concentration range of the sulfonylurea derivatives. Results are presented as means ± standard error of the mean (S.E.M.).

Table 3
Effects of sulfonylurea derivatives on flow debt repayment
Effects of vehicle, glibenclamide (Glib) and glimepiride (Glim) on the flow debt repayment are shown. Data are expressed as total coronary blood flow during the post-ischemic period (20 min) relative to the coronary blood flow at the end of baseline period (8th min). Data are presented as means ± standard error of the mean (S.E.M.).

	Flow debt repayment (a.u. ± S.E.M.)
Vehicle ($n = 8$)	3220 ± 192
0.005 μmol l^{-1} Glib ($n = 5$)	3649 ± 416
0.05 μmol l^{-1} Glib ($n = 5$)	2996 ± 322
0.25 μmol l^{-1} Glib ($n = 3$)	2461 ± 137
1 μmol l^{-1} Glib ($n = 3$)	1833 ± 149[a]
4 μmol l^{-1} Glib ($n = 5$)	1592 ± 135[b]
0.005 μmol l^{-1} Glim ($n = 5$)	3391 ± 327
0.05 μmol l^{-1} Glim ($n = 5$)	3111 ± 129
0.15 μmol l^{-1} Glim ($n = 3$)	2452 ± 238
0.25 μmol l^{-1} Glim ($n = 3$)	2045 ± 131[a]
0.8 μmol l^{-1} Glim ($n = 5$)	2146 ± 266[c]

[a] $P < 0.05$.
[b] $P < 0.001$.
[c] $P < 0.01$.

μmol l^{-1}) and −7.3 ± 0.2 (0.05 μmol l^{-1}) for glibenclamide ($P = 0.38$) and glimepiride ($P = 0.29$), respectively. Furthermore, the post-ischemic EC_{50} value tended to be higher for glibenclamide than for glimepiride ($P = 0.12$).

The flow debt repayment was significantly reduced for the two highest concentrations of both sulfonylurea derivatives (Table 3).

In the vehicle group, 12 min of global ischemia induced a calculated loss of cardiac function of 22.2 ± 2.9% (Fig. 3). The highest concentration of glibenclamide significantly reduced this ischemia-induced loss of function (4.3 ± 1.2% compared with 22.2 ± 2.5%, $P < 0.01$). At therapeutic concentrations of glibenclamide, no protective effect was observed. In contrast, low concentrations of glimepiride (0.005 and 0.05 μmol l^{-1}) significantly reduced the ischemia-induced loss of cardiac function (5.0 ± 1.4% and 4.9 ± 1.2% vs. 22.2 ± 2.9%, $P < 0.01$).

4. Discussion

This study clearly shows that both glibenclamide and glimepiride were able to significantly reduce the loss of cardiac function induced by 12 min of global ischemia. It should however be noted that glimepiride was cardioprotective at therapeutic concentrations, while for glibenclamide this protection was only present at much higher concentrations. Furthermore, this study shows that both sulfonylurea derivatives slightly reduce coronary blood flow at therapeutic concentrations. Since the majority of publications on this issue concern supratherapeutic concentrations of sulfonylurea derivatives, we think that our observations on glibenclamide and glimepiride in the therapeutic range are of special importance.

4.1. Clinical relevance

The effects of sulfonylurea derivatives on post-ischemic cardiac function are difficult to determine in a clinically relevant fashion. It is impossible to study these mechanisms in humans, so animal research is still necessary. Up to now, most studies of the cardiac effects of sulfonylurea derivatives have used concentrations that are much higher than the therapeutic range (Docherty et al., 1997; Tosaki and Hellegouarch, 1994; Ali et al., 1993; Imamura et al., 1992; Samaha et al., 1992; Mitani et al., 1991; Daut et al., 1990). 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 metabolites to the heart. However, it has been shown previously that perfusing hearts with crystalloid buffer,

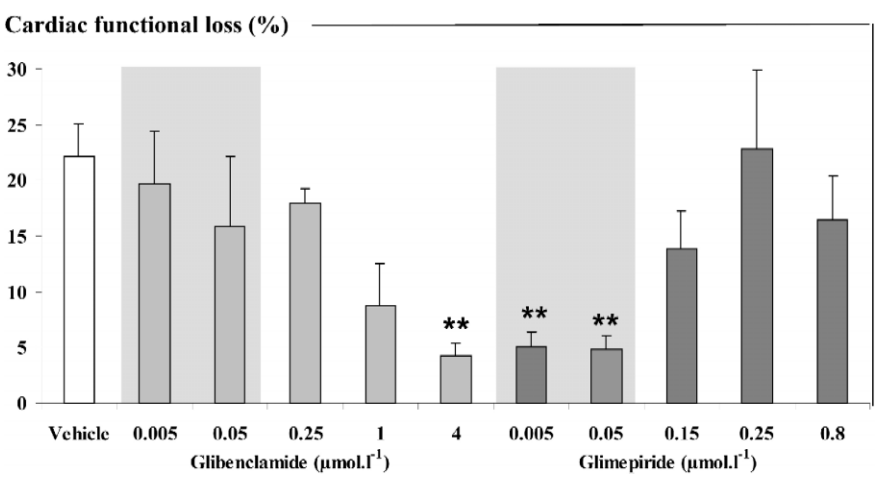

Fig. 3. Percent post-ischemic functional loss after vehicle (white bar), glibenclamide (light gray bars) or glimepiride (dark gray bars) treatment. The shaded areas represent the therapeutic range of the sulfonylurea derivatives. Results are presented as means ± standard error of the mean (S.E.M.), ** $P < 0.01$ vs. vehicle.

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 perfusion buffer to erythrocyte-enriched crystalloid buffer in our set-up (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, and also reflects the in vivo condition of patients treated with 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 fractions of the drugs also occurs in our model. Also, the isolated heart model mostly used is the so-called Langendorff heart set-up instead of the preferable working heart set-up. Because in the working heart set-up the heart actually performs external work, this model is one step further in the direction of clinical relevance than the Langendorff heart set-up.

Because of the reduced clinical relevance of the previous studies, we performed the present study with therapeutic concentrations of sulfonylurea derivatives using an isolated working rat heart set-up. In this set-up, the heart was perfused with a crystalloid buffer, enriched with erythrocytes and protein to improve the clinical relevance of observations on coronary blood flow and on functional recovery effects post-ischemically.

4.2. Hemodynamic effects

4.2.1. Coronary blood flow

The observed dose-dependent decrease in coronary blood flow after glibenclamide and glimepiride treatment during normoxia is consistent with previous observations in open-chest dogs (Imamura et al., 1992; Samaha et al., 1992). 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. Although the maximal decrease observed during the highest concentrations of glibenclamide and glimepiride was impressive, there was only slight vasoconstriction at therapeutic concentrations. Interestingly, the vasoconstrictor effect of the drugs was 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 (Noma, 1989; Coetzee, 1992). This may explain the conflicting effects in animal models that are continuously hypoxic. Consistent with the observations of Aversano et al. (1991) in anesthetized open-chest dogs, we found a decrease in the flow debt repayment with the highest concentrations of both sulfonylurea derivatives. The ischemic incident tended to decrease the calculated EC_{50}-values, suggesting that the affinity for the sulfonylurea receptor is higher after ischemia.

Interpretation of the clinical relevance of the vasoconstrictor response to both sulfonylurea derivatives on the coronary circulation 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 be underestimated in the present study.

4.2.2. Cardiac function

A high concentration of glibenclamide was found to reduce the ischemia-induced loss of cardiac function; however, therapeutic concentrations of this drug did not affect the cardiac performance. In contrast, low concentrations of glimepiride were also able to reduce the ischemia-induced loss of function. Apparently, these effects were not related to the effects on coronary blood flow. This was consistent with previous observations in isolated hearts (Docherty et al., 1997; Tosaki and Hellegouarch, 1994; Ali et al., 1993).

Other groups showed a detrimental effect on cardiac function post-ischemically in isolated hearts and isolated ventricular myocytes in the presence of sulfonylureas (Auchampach et al., 1992; Cole et al., 1991; Mitani et al., 1991). It is believed that this effect is potentiated by depolarization of the myocardial membrane followed by increased Ca^{2+} influx, thereby prolonging the ischemia-induced shortening of the action potential and increasing energy utilization, and in this way damaging the heart.

It is important to notice the difference between those ischemic insults that induce infarction, resulting in necrosis of myocardial tissue, and those that do not (e.g. stunning). This is important because sulphonylurea derivatives prevent the ischemia-induced reduction in action potential duration. As a result of a longer action potential duration, more Ca^{2+} would enter the cell and could thereby maintain contractile force in a situation of myocardial stunning. However, in a situation of myocardial infarction, the increased force development may use ATP at a faster rate and could thereby accelerate the ischemic injury.

An explanation for the improvement could be that sulfonylureas metabolically preserve high-energy phosphate compounds (Tosaki and Hellegouarch, 1994). By preserving in particular intracellular ATP levels, hearts would be less energy depleted after ischemia, and therefore they might recover better. In addition, the involvement of lactate may be important in the observed reduction in functional loss, which has been shown in isolated rat hearts (Docherty et al., 1997) and in isolated ventricular myocytes (Lederer and Nichols, 1989). Sulfonylureas may attenuate ischemia-induced intracellular lactate accumulation, implying an inhibitory effect on glycolysis and possi-

bly glycogenolysis (Docherty et al., 1997). This may be protective to the ischemic heart.

4.3. Differences in sulfonylurea derivatives

In clinical practice, type 2 diabetic patients placed on glibenclamide treatment are treated with oral doses about 2–3 times higher than those of glimepiride (15 vs. 6 mg/day maximally). These oral doses result in plasma concentrations of about 0.9 and 1.5 μmol l^{-1} for glibenclamide and glimepiride, respectively. Because sulfonylurea derivatives bind highly to protein (> 98%), the resultant non-bound concentrations are about 0.02 and 0.04 μmol l^{-1} for glibenclamide and glimepiride, respectively. Because these concentrations are peak plasma levels, we included two clinically relevant concentrations for both sulfonylurea derivatives in our experiments: 0.005 and 0.05 μmol l^{-1} (protein unbound).

Glimepiride, the newer sulfonylurea derivative, has been reported to be more specific than glibenclamide for the SUR_1 receptor in the pancreatic β cell (Bijlstra et al., 1996; Geisen et al., 1996). Geisen et al. (1996) found that a three-fold higher concentration of glimepiride is needed to close cardiovascular K_{ATP} channels to the same extent as glibenclamide. It has also been shown that glimepiride does not inhibit the protection induced by ischemic preconditioning, while glibenclamide abolishes the protection, which also supports the concept that glimepiride has fewer cardiovascular effects (Bijlstra et al., 1996). The present study, however, showed similar effects of glibenclamide and glimepiride on coronary blood flow both under normoxia and post-ischemia conditions. Therefore, there are no indications in this study that the specificity of glimepiride is different from the specificity of glibenclamide concerning vascular K_{ATP} channels or in particular the SUR_{2b} receptor. Interestingly, but hard to explain, glimepiride reduced the ischemia-induced functional loss at therapeutic concentrations, while glibenclamide did not. These observations cannot be explained by specific effects on K_{ATP} channels because at higher glimepiride concentrations, the reduced functional loss disappeared. Therefore, other mechanisms must be responsible for this effect. One possibility may be that sulfonylurea derivatives affect metabolic processes, especially during ischemia (Docherty et al., 1997; Tosaki and Hellegouarch, 1994; Lederer and Nichols, 1989).

5. Conclusion

Our observations show clearly that glibenclamide and glimepiride both reduce coronary blood flow at concentrations that are slightly higher than therapeutic ones. In the therapeutic range, the cardiac functional loss induced by 12 min of normothermic global ischemia was significantly reduced by glimepiride, while this reduction did not occur with glibenclamide. Therefore, this study suggests that both sulfonylureas, and in particular glimepiride, can be used safely in patients with type 2 diabetes mellitus, as long as the coronary vascular system is not compromised. Because of the obvious vasoconstrictor response to sulfonylureas, these drugs must be used with caution in patients with a reduced coronary reserve.

Acknowledgements

The authors would like to thank Jos Evers and Maarten de Jong for their invaluable (bio-) technical assistance during the experiments. We thank Yuen Tan for conducting the HPLC analyses. Prof. Dr. S.H. Skotnicki and Prof. Dr. F.W.A. Verheugt are acknowledged for their valuable advice. This study was supported by the Dutch Diabetes Foundation (grant 97-105) and by Hoechst Holland.

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