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Glibenclamide depletes ATP in renal proximal tubular cells by interfering with mitochondrial metabolism

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1 Sulfonylurea drugs, like glibenclamide, stimulate insulin secretion by blocking ATP-sensitive potassium channels on pancreatic beta cells. Renal tubular epithelial cells possess a different class of K_{ATP} channels with much lower affinities for sulfonylurea drugs, necessitating the use of micromolar glibenclamide concentrations to study these channels.

2 Here, we describe the toxic effects of these concentrations on mitochondrial energy metabolism in freshly isolated renal proximal tubular cells. Glibenclamide, at concentrations of 50 and 100 μM, reduced intracellular ATP levels by 28 ± 4 and $53 \pm 5\%$, respectively ($P < 0.01$).

3 This decline in ATP could be attributed to a dose-dependent inhibition of mitochondrial respiration. Glibenclamide (10–500 μM) inhibited ADP-stimulated mitochondrial oxygen consumption.

4 In addition to this toxic effect, specific association of radiolabeled and fluorescent glibenclamide to renal mitochondria was found. Association of [^{3}H]glibenclamide with renal mitochondria revealed a low-affinity site with a K_D of 16 ± 6 μM and a B_{max} of 167 ± 29 pmol mg^{-1}.

5 We conclude that micromolar concentrations of glibenclamide interfere with mitochondrial bioenergetics and, therefore, should be used with care for inhibition of epithelial K_{ATP} channels.
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Abbreviations: DNP, dinitrophenol; K_{ATP}, ATP-sensitive potassium channel; mitoK_{ATP}, mitochondrial ATP-sensitive potassium channel; RCR, respiratory control ratio; SUR, sulfonylurea receptor; TMPD, *N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine

Introduction

Sulfonylurea drugs like glibenclamide and tolbutamide are widely used in the treatment of type II diabetes. They exert their insulinotropic actions by inhibiting ATP-sensitive potassium (K_{ATP}) channels on pancreatic beta cells (Panten *et al*., 1996). These channels are metabolically regulated in such a way that a fall in intracellular ATP increases the open probability of these channels.

K_{ATP} channels were first discovered on cardiac myocytes, but have since been identified in various other tissues, for example, vascular smooth muscle cells, neurons, pancreatic beta-cells and epithelial cells like the renal tubular epithelium (Quast, 1996). These discoveries gave rise to the use of sulfonylurea drugs in experimental studies investigating the function of K_{ATP} channels in a broad range of cell types. The recent identification of mitochondrial K_{ATP} (mitoK_{ATP}) channels and their central role in ischemic preconditioning in heart muscle even boosted the use of these drugs as experimental tools (Oldenburg *et al*., 2002; Garlid *et al*., 2003; Yellon & Downey, 2003).

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The concentrations of sulfonylurea drugs needed to block ATP-sensitive potassium channels (K_{ATP}'s) in various cell types differ widely and seem to reflect the molecular composition of the K_{ATP} channel under investigation (Ashcroft & Ashcroft, 1990). K_{ATP} channels of excitable cells and pancreatic beta cells are composed of an inwardly rectifying potassium channel (Kir) and a specific sulfonylurea receptor (SUR), resulting in nanomolar inhibition by glibenclamide (Babenko *et al*., 1998). Two different genes of the receptor have been identified (Seino, 2003). SUR1 has shown to be present on pancreatic β cells, whereas SUR2 is present on cardiomyocytes, skeletal muscle (the SUR2A splice variant), and on vascular and nonvascular smooth muscles (the SUR2B splice variant). In contrast to excitable cells, renal tubular and other epithelial cells seem to be devoid of a high-affinity sulfonylurea-binding site, as reflected by the micromolar concentrations needed to inhibit these channels in patch-clamp studies (Mauerer *et al*., 1998; Tanemoto *et al*., 2000). Although the presence of renal sulfonylurea receptors has been described (SUR2A and SUR2B), it is not clear whether they are able to confer high-affinity inhibition by sulfonylurea drugs to renal potassium channels (Beesley *et al*., 1999; Tanemoto *et al*., 2000; Brochiero *et al*., 2002). Similarly, it is not known whether mitoK_{ATP} channels are regulated by a specific SUR,

although they can be inhibited by sulfonylurea drugs (Inoue *et al*., 1991).

In a previous study, we observed a protective effect of lower micromolar concentrations of glibenclamide on hypoxic tubular injury in isolated perfused rat kidney (Engbersen *et al*., 2000). However, a concentration of 100 μM glibenclamide resulted in severe damage to proximal tubules. Proximal tubules generate more than 95% of their ATP by oxidative metabolism and possess low glycolytic enzyme activity, which make them highly dependent on mitochondrial metabolism (Jones, 1986; Epstein, 1997; Lieberthal & Nigam, 1998). In this setting, intracellular ATP homeostasis is constantly threatened, as illustrated by the fact that upon stimulation of epithelial sodium transport intracellular ATP levels decline (Beck *et al*., 1991; Tsuchiya *et al*., 1992). Therefore, we expected the observed toxic effect of glibenclamide to be due to interference with mitochondrial oxidative phosphorylation. The purpose of the present study was to study the effect of glibenclamide on renal mitochondrial energy metabolism, and to investigate the binding of glibenclamide to its putative sulfonylureum receptor on isolated renal mitochondria.

Methods

Isolation of proximal tubular cells and cellular ATP determination

Proximal tubular cells (PTC) were isolated from male Wistar–Hannover rats (200–300 g) according to a method described by Boom *et al*. (1992), which results in a suspension with more than 90% proximal tubular cells and a viability above 90% as judged by trypan blue exclusion. Cells were suspended to 10–15 mg protein ml^{-1} in incubation buffer containing (mM): NaCl 117.5, KCl 4, $MgSO_4$ 1.2, KH_2PO_4 0.95, $NaHCO_3$ 22.5, glucose 11.1 and $CaCl_2$ 2.5. ATP content in proximal tubular cells was determined by a modification of Miller & Horowitz (1986) of the luciferin-luciferase procedure. Briefly, an aliquot of 1–1.5 mg cell protein was incubated in 500 μl buffer alone (control) or supplemented with various concentrations of glibenclamide. Incubation took place for 60 min at 37°C under an atmosphere of 95% oxygen and 5% carbon dioxide. Subsequently, the samples were centrifuged for 3 min at 100 × *g* and 4°C, and 100 μl 3 N perchloric acid was added to the pellet, which was then vortex-mixed, let 5 min to rest, again vortexed and centrifuged for 3 min at 100 × *g*. The sample was neutralized with 125 μl medium containing 2 N KOH, 0.4 M imidazole and 0.4 M KCl, vortex-mixed and centrifuged again. The supernatant was diluted 25 times and to 50 μl of this solution 500 μl assay medium was added, containing 50 mM glycylglycine, 7.5 mM dithiothreitol, 2 mM EGTA, 2 mM $MgCl_2$, 0.04% (w v^{-1}) bovine serum albumin (BSA), 10 μg ml^{-1} luciferin, and 2000 U ml^{-1} luciferase at pH 8.0, and measured by using a liquid scintillation counter (Beckman LS 6000 LL, U.S.A.). ATP concentrations were calculated by comparing scintillation counts with a calibration curve of various concentrations of ATP in perchloric acid : neutralization buffer 1 : 1.25. Protein content in proximal tubular cells and mitochondrial fraction was determined using the BioRad Protein Assay of BioRad (München, Germany) with BSA as the protein standard.

Isolation of renal mitochondria and respiration measurements

Male Wistar–Hannover rats (200–300 g) were used and renal mitochondria were obtained as described previously (Masereeuw *et al*., 1996b). Briefly, the kidneys were perfused with an ice-cold solution containing 140 mM NaCl and 10 mM KCl and removed. Subsequently, the medulla was dissected and the cortex was homogenized in a Potter–Elvehjem homogenizer with Teflon pestle (clearance 0.5 mm). Differential centrifugation yielded a mitochondrial pellet which was suspended to 5 mg protein ml^{-1} in respiration medium. Since isotonic or hypotonic media with low ionic strength prevent protein leakage from the mitochondria, the respiration medium had mannitol as its main component, containing (mM): mannitol 210, KCl 10, KH_2PO_4 10, EGTA 0.5, Tris-HCl 60, at pH 7.4.The low potassium concentration is unlikely to change the results since under normal conditions potassium import only contributes slightly to respiration (Garlid *et al*., 2003). Respiration measurements were carried out immediately after isolation. Oxygen consumption was measured with a Clarke-type platinum electrode, using 1 mg of mitochondrial protein in 2.0 ml of medium. Basal mitochondrial O_2 consumption was measured at 30°C in respiration medium in the absence of ADP (state 2), in the presence of 0.3 mM ADP (state 3), after ADP consumption (state 4) and after the addition of dinitrophenol (DNP; final concentration 44 μM). Succinate (10 mM) was used as the metabolic substrate, and rotenone (1 μM) was added to block electron transport proximal to succinate entry into the respiratory chain. Respiratory rates were calculated and expressed as ng atoms of oxygen per minute per milligram of protein (ng atom O min^{-1} mg prot.$^{-1}$). Glibenclamide or tolbutamide was added 2 min prior to induction of state 2 respiration by introduction of succinate. The ratio of state 3 over state 4 respiration (respiratory control ratio (RCR)) is a good indication of the coupling between oxidation and phosphorylation. Therefore, RCRs were determined directly after the isolation procedure and at the end of every experimental session to detect any decline in quality. A decline in control RCR values of >0.3 was regarded as unacceptable and measurements performed in between were not used for further analysis.

Succinate dehydrogenase assay

Since a direct effect of glibenclamide on succinate dehydrogenase has been suggested by others (Beavis *et al*., 1993), we investigated the effect of glibenclamide (0–1000 μM) on succinate dehydrogenase activity. This activity was measured according to Pennington (1961) and based on the reduction by complex II of *p*-iodo-nitro-tetrazolium-violet to *p*-iodo-nitro-tetrazolium-farmazan, which is measured spectrophotometrically at 490 nm. In addition, we measured complex i.v. activity by blocking complex III with antimycin and applying ascorbate/TMPD as an electron donor for complex IV (Kimelberg & Nicholls, 1969), which was performed in the presence of two glibenclamide concentrations (50 and 100 μM). No significant effect on succinate respiration was observed.

Specific association of glibenclamide to mitochondria

Specific association of [^{3}H]glibenclamide to rat renal cortical mitochondria in suspension (0.5 mg protein ml^{-1}) in respiration buffer was measured. Half of the suspension was pre-incubated with 500 μM glibenclamide (nonspecific association) and the other half with the solvent DMSO (total association) for 10 min at 4°C to reach equilibrium. Thereafter, 0.5 ml aliquots were taken from both suspensions and incubated with different concentrations of [^{3}H]glibenclamide (20 nM–100 μM) for 15 min at 30°C. Incubation was stopped by adding 5 ml of ice-cold respiration buffer. Associated and free [^{3}H]glibenclamide were separated by rapid filtration under vacuum over Whatman GF/C glass-fiber filters, followed by washing twice with 5 ml ice-cold respiration buffer. Subsequently, the filters were counted for associated radioactivity in a liquid scintillation system (Beckman LS 6000). Specific association was calculated by subtracting nonspecific binding from total binding.

Confocal microscopy

For confocal microscopy, cells were transferred to a Teflon chamber with a glass coverslip floor as described (Masereeuw *et al*., 1996a). In double-labeling experiments, proximal tubular cells were incubated with 0.5 μM of the mitochondrial marker tetramethylrosamine and 0.4 μM BODIPY FL glibenclamide for 30 min at room temperature. Images were obtained with an MRC 1000 confocal microscope (Bio-Rad, Hertfordshire, U.K.), coupled to a Nikon microscope equipped with an $\times 60$ objective exhibiting a numerical apperture of 1.4.

Materials

BSA was obtained from Boehringer Mannheim (Mannheim, Germany). Tolbutamide was purchased from Sigma (St Louis, MO, U.S.A.); glibenclamide, DNP and succinate were purchased from Aldrich (Zwijndrecht, The Netherlands). Both drugs were dissolved in DMSO. Tetramethylrosamine, luciferin and BODIPY FL glibenclamide were purchased from Molecular Probes (Eugene, OR, U.S.A.) and luciferase was purchased from Sigma (St Louis, MO, U.S.A.). [^{3}H]glibenclamide was obtained from New England Nuclear (NEN) Life Science Products (specific activity 50.0 Ci mmol^{-1}; 1 mCi ml^{-1}). All other chemicals were of analytical grade and purchased from Merck (Darmstadt, Germany) or Sigma (St Louis, MO, U.S.A.).

Data analysis

Data are expressed as mean $\pm$ s.e. RCR for glibenclamide and tolbutamide are presented as normalized values. For this reason, the lower limit (an RCR value of one) was set to 0% and the lowest applied glibenclamide concentration was set to 100%. This latter was done because we observed a slight but stable decline with the lowest glibenclamide concentration applied (*viz*. 1 μM glibenclamide resulted in a RCR of 2.7 compared to 3.3 for control values). The multiple means generated by respiration measurements were analyzed by using one-way analysis of variance (ANOVA), followed by post-tests with Bonferroni correction. The level of significance was set to $P<0.05$. GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, U.S.A.) was used for curve fitting. Al curves presented were best fitted by nonlinear regression analysis with a variable slope and one-binding site (Graph Pad Prism). Glibenclamide association data were best fitted according to the following formula:

$$Y = V_{max}X/(K_m + X) + kX$$

where k denotes the slope of the linear regression curve of the nonspecific association.

Results

Effect of glibenclamide on cellular ATP content

Measurement of cellular ATP by the luciferin-luciferase method yielded an ATP concentration of 2.8 ± 0.4 nmol mg prot.$^{-1}$ in control cells, comparable to earlier studies (Szewczyk & Pikula, 1998; Masereeuw *et al*., 2000). Addition of glibenclamide at a concentration of 50 or 100 μM during the incubation period reduced intracellular ATP levels by 28 ± 4 ($P<0.01$, mean $\pm$ s.e. of five different experiments) and 53 ± 5% ($P<0.001$, mean $\pm$ s.e. of five different experiments), respectively.

Effect of glibenclamide on mitochondrial respiration

To investigate the effect of glibenclamide on respiration of isolated renal tubular mitochondria, glibenclamide was added at various concentrations (ranging from 1 to 500 μM) prior to energizing the mitochondria by adding 10 mM succinate. Subsequently, oxygen consumption was measured (Table 1) prior to ADP addition (state 2), after addition of ADP (state 3) and after ADP consumption (state 4). Uncoupled respiration was measured in the presence of 44 μM DNP. The ratio of state 3 over state 4 respiration (RCR) is a good indication of the coupling between oxidation and phosphorylation. Energized mitochondria from our preparation had an RCR of 3.3 ± 0.2 ($n = 16$), indicating tight coupling and good quality.

Addition of glibenclamide resulted in a concentration-dependent decrease in state 3 (ADP-stimulated) respiration and an increase in state 4 respiration (and state 2, data not shown) that started at a concentration of 10 μM (Figure 1). Consequently, a reduction in RCR was observed in which a glibenclamide concentration of 250 μM lowered ADP-stimulated oxygen consumption to nonstimulated (state 4) levels (47 ± 5 ng atom O min^{-1} mg prot.$^{-1}$) resulting in RCR values equal to 1 (Figure 2).

Table 1 Oxygen consumption of isolated renal mitochondria

Respiratory state	*Respiratory rate* (ng atom O min^{-1} prot.$^{-1}$)
State 2	29 ± 4
State 3	97 ± 16
State 4	30 ± 4
Uncoupled	98 ± 15

State 2: before addition of ADP, state3: during ADP consumption and state 4 after ADP has been consumed. Data are presented as mean $\pm$ s.e. ($N = 16$).

The potency of sulfonylurea is a function of its affinity for the receptor. It is of interest to know whether tolbutamide, which has 100-times lower affinity for the receptor than glibenclamide, affects mitochondrial respiration. In agreement, tolbutamide showed a dose-dependent inhibition of respiration leading to a reduction of RCR values, although at much higher concentrations (Figure 2, inset). The effect of tolbutamide on respiration was much less pronounced than the effect of glibenclamide on respiration, with half-maximal inhibition coefficients of 1500 and 85 μM, respectively. To investigate whether the observed inhibition of ADP-stimulated (state 3) respiration was due to inhibition of succinate utilization, we measured succinate dehydrogenase (complex II) activity. Glibenclamide showed no effect up to a concentration of 250 μM (Figure 3), while higher concentrations showed significant inhibition of activity (IC_{50} 490 μM). In addition, no effect of glibenclamide on complex i.v. activity was seen (data not shown).

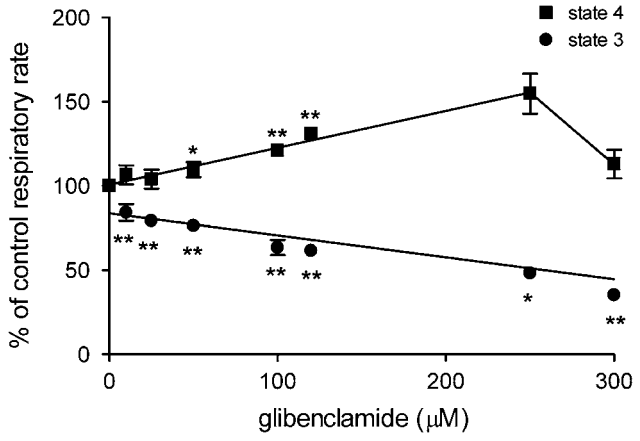

Figure 1 Respiratory rate of renal mitochondria during state 3 (●) and 4 (■) respiration with various concentrations of glibenclamide. Data are expressed as percentage of control (mean ± s.e. of nine different experiments, $*P<0.05$ and $**P<0.01$).

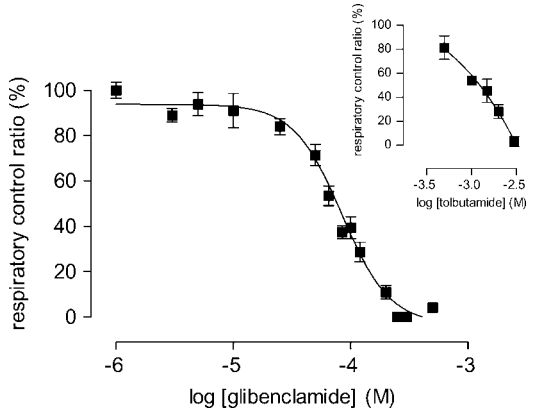

Figure 2 Decline in RCR of isolated renal mitochondria with various concentrations of glibenclamide. The inset shows the decline in RCRs of mitochondria with a tolbutamide concentration range. Data are expressed as mean ± s.e. of five independent experiments.

Association of glibenclamide to renal mitochondria

To investigate whether glibenclamide reveals specific association with isolated renal mitochondria, we performed an equilibrium assay with [^{3}H]glibenclamide. Equilibrium was reached after 10 min and remained constant over at least 30 min. We observed specific association of glibenclamide to mitochondria with a K_D of 16 ± 6 μM and a B_{max} of 167 ± 29 pmol mg^{-1} (Figure 4). Although the Scatchard insert suggests the existence of two binding sites, data were fitted best according to a one-site model.

Subsequent confocal microscopy confirmed association of the fluorescent analogue, BODIPY FL glibenclamide, in a pattern similar to the mitochondrial marker tetramethylrosamine (Figure 5).

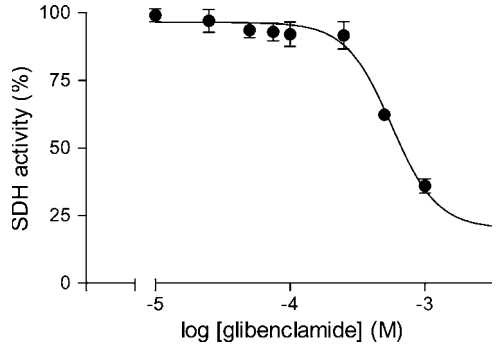

Figure 3 Influence of different concentrations of glibenclamide on succinate dehydrogenase activity. Data are expressed as percentage of control (mean ± s.e. of seven independent experiments).

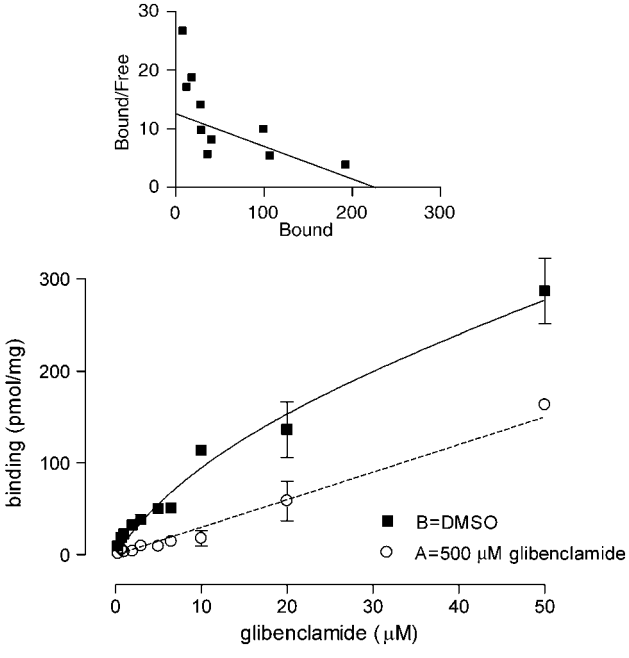

Figure 4 Association of [^{3}H]glibenclamide to isolated renal mitochondria. Data are expressed as mean ± s.e. of four independent experiments ($**P<0.01$). Specific association data were fitted best according to a one-site binding model. The inset shows a Scatchard plot. Specific association was calculated from the difference between total and nonspecific (in presence of 500 μM unlabelled glibenclamide) association.

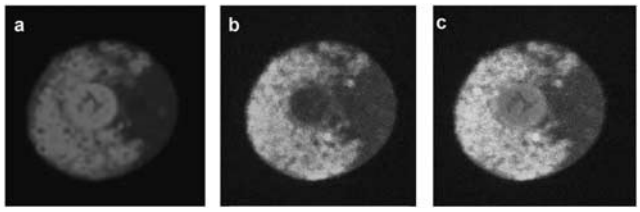

Figure 5 A representative confocal microscopic image of four different experiments with proximal tubular cells after double labeling with the mitochondrial marker (0.5 μM) tetramethylrosamine (TMRS; panel a) and (0.4 μM) BODIPY FL glibenclamide (panel b) and projection of image an over image b (panel c).

Discussion

We observed a decline in cellular ATP levels of proximal tubules, induced by micromolar concentrations of glibenclamide. Detimary *et al*. (1998) showed a decline in ATP concentrations in pancreatic islets when incubated with 100 μM tolbutamide. This fall in intracellular ATP was ascribed to a rise in $[Ca^{2+}]_i$ since it was abrogated by several measures known to prevent this rise (e.g. calcium-entry blocker nimodipine). Therefore, the mechanism by which ATP levels decline according to this study seems to be different from the mechanism described in the present study, which describes a direct effect on isolated mitochondria. White *et al*. (1988) showed a decline in ATP concentrations in rat liver mitochondria incubated with 400 μM glibenclamide and 2 mM tolbutamide. This decline was attributed to an indirect effect on metabolic flux through pyruvate carboxylase due to an uncoupling of oxidative phosphorylation. This uncoupling was demonstrated by an increase in oxygen consumption by isolated liver mitochondria and therefore is in accordance with the data presented in the present study. This decline could be attributed to a detrimental effect on mitochondrial respiration.

The respiratory chain at the mitochondrial inner membrane consists of proton pumping enzyme complexes creating an electrochemical proton gradient across the inner membrane. Subsequently, this gradient is utilized to generate ATP during the re-entry of protons into the matrix through ATP synthase. Classical uncouplers of oxidative phosphorylation are, in general, lipophilic weak acids generating a proton leak and so bypassing the ATP synthase. This proton leak results in a maximally six times increase in basal (ADP-unstimulated) respiration rate (Terada, 1990; Kadenbach, 2003).

We observed an increase in state 4 respiration, indicating uncoupling activity of glibenclamide. This effect could even be more pronounced *in vivo*, since inhibition of complex I with rotenone might result in NADH accumulation and subsequent inhibition of respiration. This view is illustrated by the fact that NADH-driven respiration results in higher RCR values than $FADH_2$-driven respiration. The observed decline in state 4 respiration seen with 500 μM glibenclamide can be attributed to the observed inhibition of succinate dehydrogenase with glibenclamide concentrations above 250 μM. The increase in state 4 respiration was less than expected for classical uncouplers. Together, with the observed decrease in state 3 respiration, this suggests an inhibition of succinate-energized respiration by glibenclamide in addition to the stimulating effect of its uncoupling properties. Similar effects on mitochondrial respiration have been reported for drugs that can be grouped as lipophilic amines. These drugs are able to enter the mitochondrial matrix along an electrically favorable gradient in their protonated cationic form (Fromenty *et al*., 1990; Deschamps *et al*., 1994; Berson *et al*., 1996).

In the case of mitochondria respiring on succinate, there are theoretically several explanations for the effect of glibenclamide on respiration: (1) inhibition of succinate uptake; (2) inhibition of the adenine nucleotide translocase (ANT); (3) disturbed electron flow by inhibition of complex II, III or IV activity and (4) reversal of the ATP synthesis function of F_0F_1-ATPase leading to ATP hydrolysis and extrusion of protons out of the mitochondrial matrix.

Inhibition of succinate uptake is a plausible explanation since the increased respiration during state 3 requires rapid formation of $FADH_2$ and, therefore, substrate (succinate) availability. This makes state 3 more vulnerable to glibenclamide inhibition as compared to state 4 respiration. However, we used a succinate concentration a 100-fold higher than the apparent K_M of the succinate (dicarboxylate) carrier of mitochondrial inner membrane (Pallotta *et al*., 1999), making an effect of micromolar concentrations of glibenclamide on succinate transport unlikely.

Inhibition of ANT cannot be excluded as a possible mechanism although glibenclamide is structurally different from the two known classes of inhibitors of ANT, which demonstrate a very narrow structural specificity (Fiore *et al*., 1998).

Beavis *et al*. (1993) suggested direct inhibition of succinate dehydrogenase (complex II) by micromolar concentrations of glibenclamide. However, we observed no inhibitory effect of glibenclamide on complex II activity up to concentrations of 250 μM. Also, no inhibitory effect was observed on complex i.v. activity with 50 and 100 μM glibenclamide (data not shown). This was confirmed by a study of Jaburek *et al*. (1998), who also showed no inhibition of complex i.v. activity by glibenclamide in isolated mitochondria of heart and liver. Together, these observations leave inhibition of complex III activity still open as a possible mechanism for the observed inhibition. However, the mitochondrial effects of glibenclamide are best described as a DNP-like effect. This classical uncoupler stimulates reversal of the ATP synthase, which counteracts the effect of ADP added to respiring mitochondria resulting in inhibition of state 3 respiration. Indeed, Debeer *et al*. (1974) showed such stimulation of the ATPase activity of ATP synthase by different sulfonylurea compounds.

Although the present results gave no definitive explanation of the mechanism behind the observed inhibition of mitochondrial respiration, our data may have important implications for research on epithelial K_{ATP} channels since micromolar concentrations of sulfonylurea drugs are needed for the inhibition of these channels. This concern is illustrated by several studies in which inhibition of mitochondrial metabolism resulted in activation of K_{ATP} channels (Leyssens *et al*., 1996; Abe *et al*., 1999; Sasaki *et al*., 2001). Roper & Ashcroft (1995) demonstrated in neurons that rotenone induced inhibition of complex I of the respiratory chain generated a potassium current at the plasma membrane which could be inhibited by ATP and sulfonylurea drugs.

In addition to its detrimental effects on respiration, we observed a low-affinity association site for glibenclamide on renal mitochondria, indicating the existence of renal mitoK_{ATP}

channels. To date, not much is known about the molecular identity of these channels, but they can be inhibited by sulfonylurea drugs (Inoue *et al*., 1991). Binding studies on heart and liver submitochondrial particles suggest the existence of a SUR, although of smaller size than its counterpart in plasma membranes (Szewczyk *et al*., 1996; 1997). Szewczyk *et al*. (1997) found [^{3}H]glibenclamide-binding sites on bovine heart submitochondrial particles with a dissociation constant of 360 nM. Photoaffinity labeling with [^{125}I]glibenclamide revealed a protein with a molecular size of 28 kDa. Rat liver submitochondrial particles demonstrated binding of [^{3}H]glibenclamide with a K_D of 4 μM. We showed association of [^{3}H]glibenclamide to intact renal mitochondria with a somewhat higher K_D of 16 μM. Binding of amphiphilic molecules like glibenclamide may be influenced by the lipid and protein composition of mitochondrial membranes, which are known to be different in mitochondria from various tissues. Glibenclamide uptake cannot be excluded since we used intact mitochondria. However, no specific transporters for glibenclamide have been described and the reported K_D is in the same range as in earlier reports that used submitochondrial particles in which specific transport can be ruled out. The molecular identity of mitoK_{ATP} channels remains unknown; nevertheless, the present description of glibenclamide binding to renal mitochondria is in accordance with a recent report showing ATP-sensitive and glibenclamide-inhibited potassium fluxes in renal mitochondria (Cancherini *et al*., 2003). There is strong evidence that mitoK_{ATP} channels are closed under normal conditions but open during ischemia (Garlid & Paucek, 2003; Garlid *et al*., 2003). Due to dissipation of mitochondrial membrane potential, ischemia results in a reduction of potassium leakage, leading to a contraction of the mitochondrial matrix. Opening of mitoK_{ATP} might counteract this reduced potassium leakage, thereby maintaining the mitochondrial volume, which is essential for normal functioning (Garlid & Paucek, 2003; Garlid *et al*., 2003). Binding of glibenclamide to mitoK_{ATP} channels and closure of these channels under ischemic conditions might, therefore, aggravate injury.

In summary, glibenclamide affects mitochondria in two ways: (1) by interfering with mitochondrial bioenergetics, resulting in a loss of cellular ATP, and (2) by specific binding to mitochondria, possibly to renal mitoK_{ATP} channels. These findings urge us to warn for the potential harmful effects of micromolar concentrations of glibenclamide when used in research on epithelial K_{ATP} channels.

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