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State-dependent Inhibition of the Mitochondrial K_{ATP} Channel by Glyburide and 5-Hydroxydecanoate*

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The mitochondrial K_{ATP} channel (mitoK_{ATP}) is hypothesized to be the receptor for the cardioprotective effects of K^+ channel openers (KCO) and for the blocking of cardioprotection by glyburide and 5-hydroxydecanoate (5-HD). Studies on glyburide have indicated that this drug is inactive in isolated mitochondria. No studies of the effects of 5-HD on isolated mitochondria have been reported. This paper examines the effects of glyburide and 5-HD on K^+ flux in isolated, respiring mitochondria. We show that mitoK_{ATP} is completely insensitive to glyburide and 5-HD under the experimental conditions in which the open state of the channel is induced by the absence of ATP and Mg^{2+}. On the other hand, mitoK_{ATP} became highly sensitive to glyburide and 5-HD when the open state was induced by Mg^{2+}, ATP, and a physiological opener, such as GTP, or a pharmacological opener, such as diazoxide. In these open states, glyburide ($K_{½}$ values 1–6 μM) and 5-HD ($K_{½}$ values 45–75 μM) inhibited specific, mitoK_{ATP}-mediated K^+ flux in both heart and liver mitochondria from rat. These results are consistent with a role for mitoK_{ATP} in cardioprotection and show that different open states of mitoK_{ATP}, although catalyzing identical K^+ fluxes, exhibit very different susceptibilities to channel inhibitors.

During steady-state respiration, K^+ influx into mitochondria is balanced by K^+ efflux on the K^+/H^+ antiporter, and steady-state volume is maintained. Opening the mitochondrial K_{ATP} channel (mitoK_{ATP})[1] will increase K^+ influx into mitochondria and shift matrix volume to a higher steady state. The energetic costs of this futile cycle are small, between 100–150 nmol of H^+/mg of protein·min at 25 °C, and we have concluded that the sole function of mitoK_{ATP} is to regulate matrix volume (1). It has been suggested that matrix expansion secondary to mitoK_{ATP} opening plays an important role in cell-signaling pathways calling for activation of electron transport and stimulation of fatty acid oxidation (2).

A pharmacological role for mitoK_{ATP} in cardiac ischemia seems clearer. During prolonged cardiac ischemia, myocyte ATP levels fall, and the heart does not survive reperfusion. Either pretreatment with K^+ channel openers (KCO) or preconditioning with a brief period of ischemia protects the heart; during subsequent ischemia, ATP loss is reduced, and the heart recovers to nearly normal function upon reperfusion (3). Importantly, cardioprotection by either KCO or preconditioning is blocked by glyburide and 5-HD. This set of agents, KCO, glyburide, and 5-HD, identifies the receptor as a K_{ATP} channel, and pharmacological studies indicate that mitoK_{ATP} is the receptor for these effects (4).

A major problem with this hypothesis has been that glyburide appears to be ineffective as a specific inhibitor of K^+ flux in intact, respiring mitochondria (5). Nonspecific inhibition of K^+ flux does occur (5); however, we have attributed this effect, which occurs at high doses of glyburide, to low affinity reactions with key energy-transducing enzymes (6–8). The effect of these nonspecific actions is to reduce $\Delta\Psi$, the driving force for K^+ uptake, and has nothing to do with mitoK_{ATP}. We have now verified this conclusion in experiments that examine the effects of glyburide on both respiration and respiration-driven cation uptake into mitochondria.

Failure to inhibit mitoK_{ATP} seemed to be a property of both glyburide and 5-HD. We recognized, however, that these drugs had only been studied under conditions when no other ligands of mitoK_{ATP} were present, a condition that never obtains *in vivo*. We now report that glyburide and 5-HD are potent blockers of K^+ flux through mitoK_{ATP} in open states in which Mg^{2+}, ATP, and physiological (GTP) or pharmacological (KCO) openers are present. In intact rat heart mitochondria, $K_{½}$ values for glyburide and 5-HD are about 1 and 50 μM, respectively. We infer that susceptibility to glyburide and 5-HD requires a ligand-induced conformational change in the mitochondrial sulfonylurea receptor (mitoSUR). These results are consistent with a role for mitoK_{ATP} in cardioprotection (4, 9).[2]

EXPERIMENTAL PROCEDURES

Preparations—Rat liver mitochondria were prepared according to Pedersen *et al.* (10), and rat heart mitochondria were prepared by the Glass-Teflon™ homogenization procedure according to Matlib *et al.* (11). The final mitochondrial pellet was washed and resuspended at 50 mg/ml (liver) or 20 mg/ml (heart) in 0.22 M mannitol, 0.07 M sucrose, and potassium salts of 5 mM TES and 0.5 mM EGTA. Mitochondria were kept on ice at pH 7.2 during the experiments. MitoK_{ATP} was purified and reconstituted as described previously (12).

Assay of Ion Transport in Intact Mitochondria—K^+ or TEA^+ uptake

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[1] The abbreviations used are: mitoK_{ATP}, mitochondrial ATP-sensitive potassium channel; KCO, potassium channel opener(s); 5-HD, 5-hydroxydecanoate; PBFI, potassium-binding benzofuran isophthalate; TEA^+, tetraethylammonium cation; TES, *N*-tris(hydroxymethyl) methyl-2-aminoethanesulfonic acid; TMPD, *N,N,N,N′*-tetramethyl-*p*-phenylenediamine; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; CCCP, carbonyl cyanide *p*-chlorophenylhydrazone; mitoSUR, mitochondrial sulfonylurea receptor.

[2] A preliminary account of these findings was reported in abstract form (26).

was assayed by following swelling, which accompanies net salt transport, using previously established light-scattering techniques (13, 14). Reciprocal absorbance (A^{-1}) at 520 nm varies linearly with matrix volume within three well defined regions, separated by transitions at 115 and 68 milliosmolal (13). β is a dimensionless parameter that normalizes A^{-1} for mitochondrial protein concentration, P (mg/ml),

$$\beta \equiv P/P_s(A^{-1} - a), \quad \text{(Eq. 1)}$$

where a is a machine constant (0.25 for our apparatus), and P_s equals 1 mg/ml.

To obtain initial rates ($d\beta/dt$), it is necessary to avoid the sharp discontinuities in the relationship between β and matrix volume. For ion flux measurements, we normally begin measurements at 115 milliosmolal, where the outer membrane begins to break (14). This technique is particularly important for light scattering of rat heart mitochondria, which is only weakly sensitive to volume changes in the isosmotic domain. Thus, kinetic curves in 250 milliosmolal salts exhibit an artifactual "lag" during salt uptake as volume goes through the first transition point (14).

115 milliosmolal assay media contained either K^+ or TEA^+ salts of chloride (45 mM), acetate (25 mM), EGTA (0.1 mM), and TES (5 mM), pH 7.4. Media also contained $MgCl_2$ (0.1 mM), rotenone (5 μg/mg), and cytochrome *c* (10 μM). Respiratory substrates were either succinate (3 mM) or ascorbate (2.5 mM) plus TMPD (0.25 mM). Mitochondria were assayed at a concentration of 0.1 mg of protein/ml at 25 °C.

Measurement of Respiration—Respiration was measured with a Yellow Springs oxygen electrode assembly in a hypotonic assay medium identical to the one used for measurement of K^+ uptake, or in an isotonic assay medium consisting of K^+ salts of chloride (120 mM), succinate (10 mM), phosphate (5 mM), TES (5 mM), and $MgCl_2$ (0.1 mM), pH 7.4, supplemented with rotenone (2 μg/mg of protein). Mitochondria were assayed at 1 mg of protein/ml at 25 °C.

Assay of K^+ Flux in Liposomes—$MitoK_{ATP}$ was partially purified from rat liver mitochondria and reconstituted into proteoliposomes loaded with PBFI according to previously published protocols (15). *Internal medium* contained TEA^+ salts of sulfate (100 mM), EGTA (1 mM), and HEPES (25 mM) at pH 6.8. 15 μl of stock proteoliposomes (50 mg of phospholipids/ml) were added to 2 ml of *external medium* containing 150 mM KCl and TEA^+ salts of EGTA (1 mM) and HEPES (25 mM), at pH 7.4. Temperature was maintained at 25 °C during assays. Electrophoretic K^+ flux was initiated by the addition of 1 μM FCCP to provide charge compensation and measured from changes in PBFI fluorescence (12).

Chemicals and Reagents—PBFI was obtained from Molecular Probes, Inc.; HEPES was obtained from Calbiochem; 5-HD was obtained from Research Biochemicals Inc.; and other drugs and chemicals were obtained from Sigma. The Tris salt of ATP was titrated to pH 7.2 with Tris base.

RESULTS

Specific and Nonspecific Cation Flux in Respiring Heart Mitochondria—Respiring mitochondria take up K^+ by nonspecific and specific mechanisms, *i.e. via* diffusion and *via* $mitoK_{ATP}$. We used TEA^+ to distinguish between these two parallel mechanisms, as demonstrated by the four traces in Fig. 1*A*. K^+ flux (*trace a*) is greater than TEA^+ flux (*trace b*). Addition of ATP inhibits K^+ flux (*trace c*) but not TEA^+ flux (*trace d*). These results, which are routinely observed in rat heart (Fig. 1) and liver (5) mitochondria, permit the following conclusions: (i) TEA^+ is transported solely by diffusive leak (16) and is not transported by $mitoK_{ATP}$; and (ii) in the presence of ATP, K^+ is transported solely by diffusion, and its flux equals that of TEA^+. Thus, TEA^+ flux may be used as a control for the component of K^+ flux due to leak.

The traces in Fig. 1*B* show that 10 μM glyburide does not inhibit K^+ flux under these conditions, despite the fact that $mitoK_{ATP}$ is evidently open. 100 μM 5-HD was similarly ineffective (data not shown). To summarize a large body of experiments, these agents did not inhibit K^+ flux at these doses in rat liver or heart mitochondria respiring on succinate or TMPD/ascorbate. This failure of glyburide and 5-HD to inhibit is the central problem addressed by this paper.

Nonspecific Effects of Glyburide on K^+ Flux in Mitochondria—In massive doses, glyburide does inhibit K^+ flux, as previously reported by us (5) and by Belyaeva *et al.* (17) and Szewczyk *et al.* (18). We concluded, however, that this effect was secondary to inhibition of respiration (5). Cation uptake into respiring mitochondria is exquisitely sensitive to $\Delta\Psi$ (16), and it is essential to differentiate between nonspecific inhibition due to reduced driving force and specific inhibition of $mitoK_{ATP}$. Accordingly, we examined the effect of glyburide on uncoupled respiration, on respiration-driven K^+ and TEA^+ uptake, and on respiration-driven K^+ uptake in the presence of valinomycin.

The dose-response curves in Fig. 2 confirm that glyburide inhibits succinate utilization during uncoupled respiration. In hypotonic assay medium supplemented with acetate and cyto-

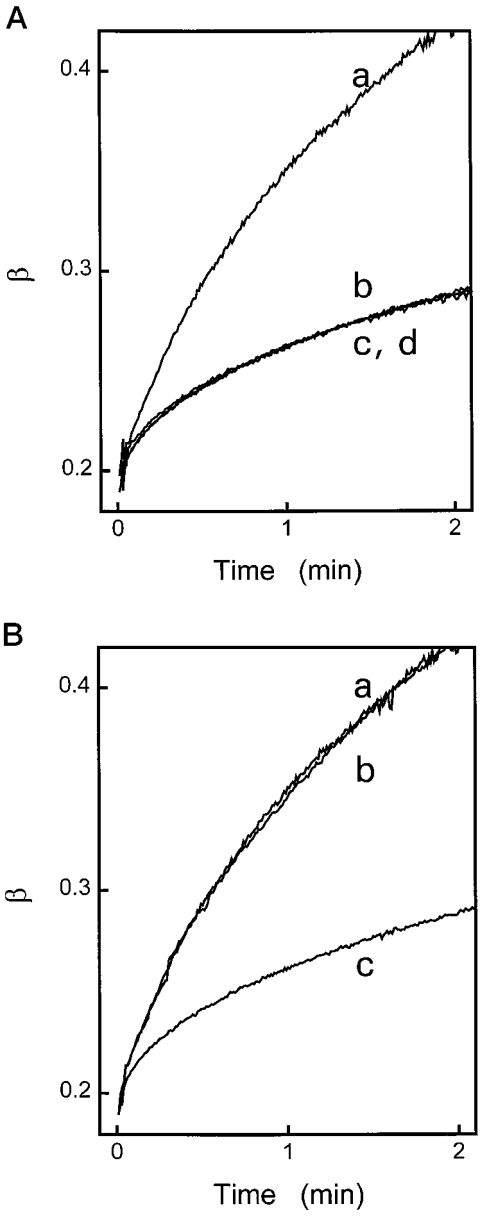

FIG. 1. **ATP-dependent K^+ uptake by mitochondria.** Light-scattering traces from rat heart mitochondria respiring on ascorbate/TMPD in K^+ or TEA^+ medium, as described under "Experimental Procedures." *A*, $mitoK_{ATP}$ is K+ selective: *trace a*, K^+ influx in the absence of ATP; *trace b*, K^+ influx in the presence of 0.2 mM ATP; *trace c*, TEA^+ influx in the absence of ATP; and *trace d*, TEA^+ influx in the presence of 0.2 mM ATP. *B*, failure of glyburide to inhibit K+ influx: *trace a*, K^+ influx in the absence of ATP; *trace b*, K^+ influx in the presence of 10 μM glyburide; and *trace c*, K^+ influx in the presence of 0.2 mM ATP.

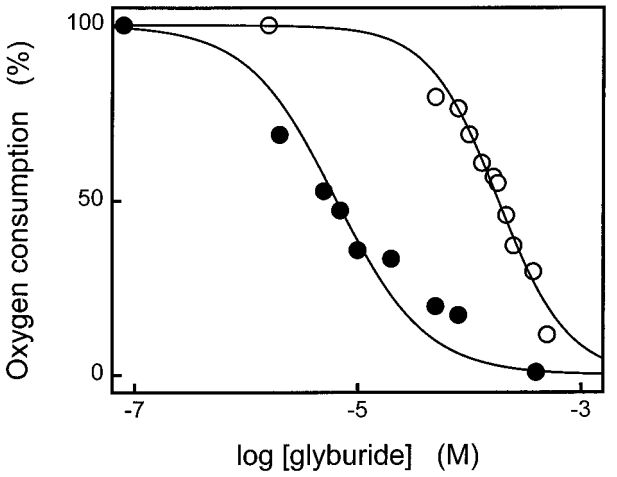

FIG. 2. **Glyburide inhibits respiration of rat liver and rat heart mitochondria.** Oxygen consumption of rat heart (●) and rat liver (○) mitochondria (1 mg of protein/ml) respiring on succinate is plotted *versus* medium glyburide concentration. Mitochondria were uncoupled by addition of 0.25 μM CCCP.

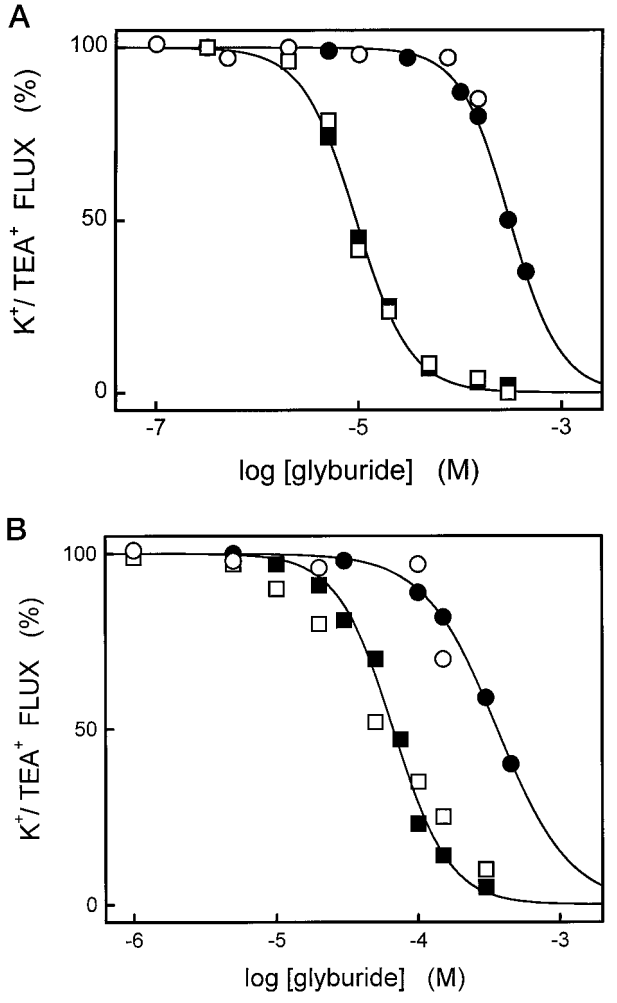

FIG. 3. **Glyburide nonspecifically inhibits cation flux into mitochondria.** Dose-response curves for glyburide inhibition of respiration-driven mitochondrial swelling in K^+ (○, □) and TEA^+ (●, ■) media. K^+ and TEA^+ were inhibited identically in both succinate (□, ■) and ascorbate/TMPD (○, ●). *A*, rat heart mitochondria; *B*, rat liver mitochondria.

chrome *c*, the $K_{½}$ values for inhibition were 5.7 ± 2.7 μM (n = 3) in rat heart mitochondria and 157 ± 27 (n = 3) in rat liver mitochondria. In isotonic assay medium without acetate, the

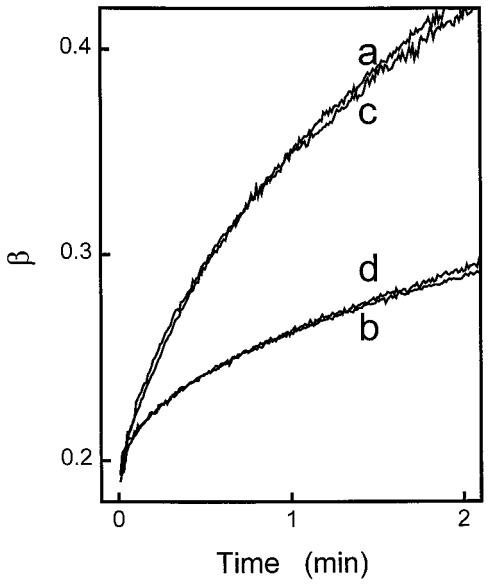

FIG. 4. **Glyburide inhibits the pharmacological open state of mitoK$_{ATP}$.** Light-scattering traces from rat heart mitochondria respiring on ascorbate/TMPD in K^+ medium. *Trace a*, K^+ influx in the absence of ATP; *trace b*, K^+ influx in the presence of 0.2 mM ATP; *trace c*, reversal of inhibition by 10 μM diazoxide in the presence of 0.2 mM ATP; and *trace d*, reinhibition by 10 μM glyburide in the presence of 10 μM diazoxide and 0.2 mM ATP.

$K_{½}$ values for inhibition were 5.3 ± 0.5 μM (n = 2) in rat heart mitochondria and 70 ± 2 μM (n = 2) in rat liver mitochondria (not shown).

A more direct demonstration of the nonspecific effect of glyburide on K^+ uptake was obtained in protocols measuring respiration-dependent cation uptake. As shown in Fig. 3, cation uptake was inhibited by glyburide as a function of dose. In rat heart mitochondria (Fig. 3*A*), the $K_{½}$ values were 6.4 ± 1.1 μM (n = 3) when succinate was used as substrate and 470 ± 35 μM (n = 2) when ascorbate/TMPD was used as substrate. In rat liver mitochondria (Fig. 3*B*), the $K_{½}$ values were 63 ± 13 μM (n = 3) when succinate was used as respiratory substrate and 476 ± 34 μM (n = 3) when ascorbate/TMPD was used as substrate. Inhibition was nonselective—K^+ flux and TEA^+ flux were inhibited at the same doses—and the $K_{½}$ values were essentially the same as $K_{½}$ values for respiratory inhibition (Fig. 2). These findings demonstrate that inhibition of cation flux was due to reduction in driving force and not due to inhibition of mitoK$_{ATP}$. Similar results were obtained for valinomycin-induced swelling in potassium medium (data not shown).

We conclude that the only inhibition mediated by glyburide under these conditions is nonspecific, secondary to inhibition of respiration and a reduction of the driving force for cation uptake.

Nonspecific Effects of 5-HD on K^+ Flux in Mitochondria—An identical series of experiments was carried out using 5-HD. 5-HD had no effect on K^+ flux under these conditions. In contrast to glyburide, 5-HD did not inhibit uncoupled respiration or cation uptake in either rat liver or heart mitochondria up to 500 μM (data not shown).

Open States in which Glyburide and 5-HD Specifically Inhibit Rat Heart MitoK$_{ATP}$—Under the conditions of Fig. 1, mitoK$_{ATP}$ is open because no inhibitory ligands are present. *In vivo*, mitoK$_{ATP}$ would be opened by pharmacological agents (KCO), such as diazoxide or cromakalim (15), or physiological ligands, such as GTP (12). Moreover, ATP and Mg^{2+} would also be present *in vivo*. When mitochondria were studied under these more physiological conditions, glyburide and 5-HD were

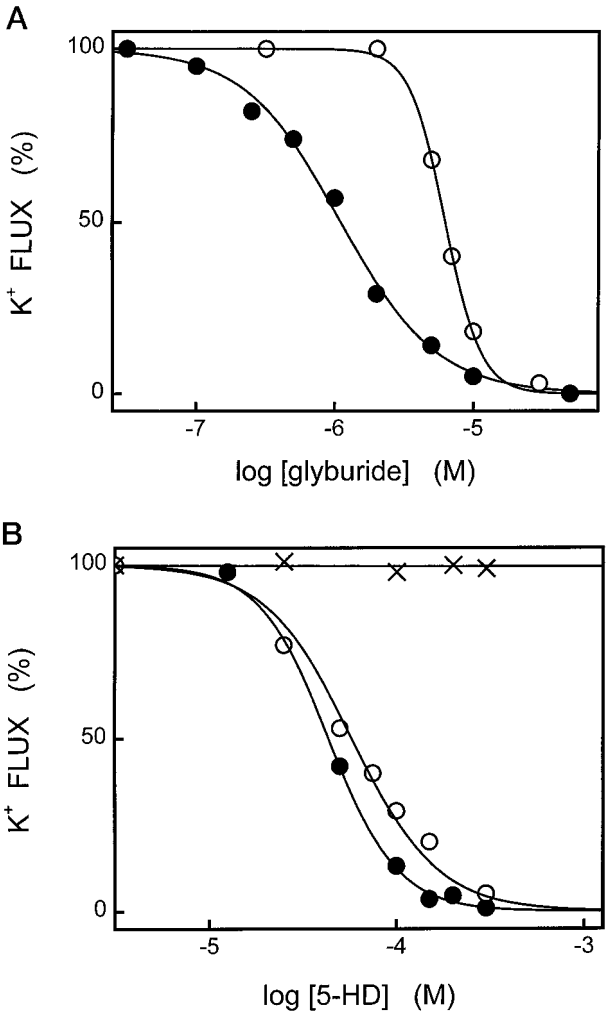

FIG. 5. **Specific inhibition of K^+ influx in rat heart mitochondria by glyburide and 5-HD.** K^+ influx, estimated from the light-scattering assay, was measured in mitochondria respiring on ascorbate/TMPD in K^+ medium containing 0.1 mM Mg^{2+}. *A*, inhibition by glyburide. Dose-response curves were generated in the presence of pharmacological opener (●), containing 0.2 mM ATP plus 10 μM diazoxide, and in the presence of physiological opener (○), containing 0.2 mM ATP plus 50 μM GTP. *B,* inhibition by 5-HD. Dose-response curves were generated in the presence of pharmacological opener (●), containing 0.2 mM ATP plus 10 μM diazoxide, and in the presence of physiological opener (○), containing 0.2 mM ATP plus 50 μM GTP. In the absence of ATP (×), 5-HD failed to inhibit. Rates of ATP-dependent K^+ influx were determined from light-scattering traces similar to those shown in Fig. 4.

potent, specific inhibitors of K^+ flux, as illustrated by the traces in Fig. 4. Control K^+ flux (Fig. 4, *trace a*) was inhibited in the presence of ATP and Mg^{2+} (Fig. 4, *trace b*) and then restored to control values by diazoxide (Fig. 4, *trace c*). This pharmacologically induced K^+ flux was strongly inhibited by 10 μM glyburide to the level associated with ATP inhibition (Fig. 4, *trace d*). Note that this glyburide dose had no effect on respiration in ascorbate/TMPD and no effect on TEA^+ flux (see Fig. 3*A*). Therefore, the effect is specific for K^+ flux through mitoK_{ATP}.

Fig. 5, *A* and *B,* contains the concentration dependences of specific glyburide and 5-HD inhibition of K^+ flux. The opening effect of 10 μM diazoxide was reversed by glyburide with $K_{½}$ = 1.1 μM (closed circles in Fig. 5*A*), and by 5-HD with $K_{½}$ = 45 μM (closed circles in Fig. 5*B*). The opening effect of 50 μM GTP was reversed by glyburide with $K_{½}$ = 6 μM (Fig. 5*A, open circles*), and by 5-HD with $K_{½}$ = 58 μM (Fig. 5*B, open circles*).

Three additional ligands were required for specific glyburide inhibition of mitoK_{ATP}: Mg^{2+}, ATP, and either GTP or a KCO.

TABLE I

Specific inhibition of mitoK_{ATP} by glyburide and 5-HD

The table compares the mean values of half-maximal inhibition (±S.D., n = 3) by glyburide and 5-HD in three different preparations. Intact mitochondria were respiring on succinate or ascorbate/TMPD in K^+ medium, and K^+ influx was estimated from the light scattering assay. Assay media for mitochondria contained varying doses of glyburide or 5-HD and 0.1 mM Mg^{2+}. When added, ATP was 0.2 mM. K^+ flux through reconstituted rat liver mitoK_{ATP} was determined from fluorescence of PBFI. Assay media for liposomes contained varying doses of glyburide or 5-HD and 0.5 mM Mg^{2+}. When added, ATP was 0.5 mM. ND, not determined.

Experimental preparation	$K_{1/2}$-glyburide	$K_{1/2}$-5-HD
Rat heart mitochondria		
No ATP	No effect	No effect
ATP + 10 μM diazoxide	1.1 ± 0.2 μM	45 ± 5 μM
ATP + 50 μM GTP	6 ± 1 μM	58 ± 5 μM
Rat liver mitochondria		
No ATP	No effect	No effect
ATP + 10 μM cromakalim	3.4 ± 0.3 μM	73 ± 3 μM
Reconstituted mitoK_{ATP}		
No ATP	250 ± 15 nM	No effect
ATP + 10 μM cromakalim	90 ± 6 nM	85 ± 5 μM
ATP + 20 μM GTP	80 ± 5 nM	ND

No single one of these, nor any combination of two, was effective.

Open States in Which Glyburide and 5-HD Specifically Inhibit Rat Liver MitoK_{ATP}—An identical series of experiments was carried out with rat liver mitochondria, and the results were qualitatively identical to the results with rat heart mitochondria. We were also able to compare the effect of substrates on glyburide potency in liver mitochondria because the margin of safety for nonspecific inhibition during respiration on succinate is much wider in liver than in heart mitochondria (compare Fig. 3, *A* and *B*). The results are summarized in Table I. The $K_{½}$ for glyburide inhibition was 3.4 μM when either succinate or ascorbate/TMPD was the respiratory substrate. 5-HD also inhibited cromakalim-opened mitoK_{ATP} in liver mitochondria respiring on either succinate or ascorbate/TMPD with equal potency ($K_{½}$ = 73 μM).

Specific Inhibition of Reconstituted MitoK_{ATP} by Glyburide and 5-HD—Table I also summarizes the effects of glyburide and 5-HD on reconstituted mitoK_{ATP} purified from rat liver mitochondria. In the presence of Mg^{2+}, ATP, and cromakalim, both glyburide and 5-HD inhibited mitoK_{ATP}. Glyburide inhibited with $K_{½}$ = 90 nM, and 5-HD inhibited with $K_{½}$ = 85 μM. In the absence of ATP, 5-HD had no effect, but glyburide inhibited K^+ flux with $K_{½}$ = 250 nM. The latter result is consistent with our previous results on reconstituted mitoK_{ATP} in the presence of Mg^{2+} (19). Glyburide was also a potent inhibitor ($K_{½}$ = 80 nM) of the reconstituted mitoK_{ATP} in the presence of Mg^{2+}, ATP, and GTP.

DISCUSSION

There is growing evidence for the hypothesis that mitoK_{ATP} is the receptor for the cardioprotective actions of K^+ channel openers and the cardio-damaging actions of glyburide and 5-HD (4, 9). Glyburide is a prototypical sulfonylurea inhibitor that acts on all K_{ATP} channels and blocks the protective effects of both KCO and cardiac preconditioning (20, 21). 5-HD, which is structurally unrelated to glyburide, has been characterized as an ischemia-selective inhibitor of K_{ATP} channels (22, 23). Like glyburide, 5-HD selectively blocks the protective effects of both KCO (22) and cardiac preconditioning (24, 25). There have been no previous studies of the effects of 5-HD on mitochondria. The hypothesis that mitoK_{ATP} is involved in cardiac protection has been clouded by the lack of a crucial piece of evidence: a convincing demonstration that glyburide and 5-HD inhibit ATP-dependent K^+ uptake in intact mitochondria.

MitoK$_{ATP}$ activity can readily be elicited in respiring mitochondria, as demonstrated by the data in Fig. 1. We showed previously that glyburide inhibits K^+ flux under these conditions; however, inhibition required high doses that inhibit respiration in the presence of succinate, and we concluded that this inhibition was nonspecific (5). Belyaeva *et al.* (17) concluded to the contrary that mitoK$_{ATP}$ was specifically inhibited by 150 μM glyburide. To resolve this disagreement, we carried out a thorough study of the nonspecific effects of glyburide, as reported under "Results." We found that glyburide is a potent inhibitor of uncoupled respiration in both liver and heart mitochondria when succinate was used as the respiratory substrate (Fig. 2). Over the same dose range, glyburide also inhibited diffusive K^+ and TEA^+ fluxes (Fig. 3). TEA^+ is transported solely by leak pathways; accordingly, glyburide inhibited K^+ and TEA^+ fluxes by depression of $\Delta\Psi$ and not by inhibition of mitoK$_{ATP}$. Thus, glyburide inhibition of K^+ flux under these conditions is nonspecific.

Respiration using ascorbate/TMPD is relatively insensitive to inhibition by glyburide and 5-HD, and the flux experiments using these substrates (Fig. 3) permit a further conclusion: glyburide and 5-HD, *at any dose*, are completely ineffective under the conditions that have routinely been used to study inhibition of K^+ flux through mitoK$_{ATP}$, namely respiration on succinate with rotenone.

These findings presented a serious obstacle to studies of the pharmacological regulation of mitoK$_{ATP}$ in mitochondria. We finally recognized, however, that the conditions routinely used, namely, in the absence of other ligands of mitoK$_{ATP}$, are far from those present *in vivo*. In a living cell, the open channel would never be exposed to glyburide under such conditions. Rather, the channel would be exposed to ATP and Mg^{2+} and then opened by GTP or a K^+ channel opener (12, 15). Indeed, when the *in vitro* experiments were adjusted to mimic these *in vivo* conditions, we found glyburide and 5-HD to be potent, specific blockers of K^+ flux in the open states induced by physiological or pharmacological ligands (Figs. 4 and 5 and Table I). We emphasize that three components, Mg^{2+}, ATP, and a physiological or pharmacological opener, were required to achieve inhibition by either of these drugs. No single component nor any combination of two components was sufficient. This phenomenon was observed in both heart and liver mitochondria.

Results with reconstituted mitoK$_{ATP}$ qualitatively reflect results in intact mitochondria (Table I). With glyburide, the $K_{½}$ value was reduced when studied in the pharmacological open state. With 5-HD, no inhibition was observed in the absence of ATP, but 5-HD inhibited in the pharmacological open state, when ATP and a KCO were present.

Some aspects of these findings raise new scientific questions. (i) What renders mitoK$_{ATP}$ susceptible to these inhibitors in one open state and not the other? In view of the fact that K^+ fluxes are identical in all open states, we conclude that the protein must be in a different state. Thus, we infer that Mg^{2+}, ATP, and an opener induce a conformation in mitoSUR that renders it susceptible to glyburide and 5-HD. (ii) Why is glyburide a potent inhibitor of reconstituted mitoK$_{ATP}$ under conditions in which it is ineffective in intact mitochondria? The logical inference is that mitochondria are regulated by a factor that is lost during reconstitution. These hypotheses are under investigation.

In conclusion, our results are consistent with the hypothesis that mitoK$_{ATP}$ is the essential drug receptor involved in ischemic cardioprotection. They also remove the previous deterrent to pharmacological studies on mitoK$_{ATP}$ in intact mitochondria.

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