Pharmacological and Histochemical Distinctions Between Molecularly Defined Sarcolemmal K<sub>ATP</sub> Channels and Native Cardiac Mitochondrial K<sub>ATP</sub> Channels

HAI HU, 1, 2 TOSHIKIKI SATO, 1 JEGATHEESAN SEHBARASEYON, YONGGE LIU, 3 DAVID C. JOHNS, BRIAN O’ROURKE, and EDUARDO MARBAN

Section of Molecular and Cellular Cardiology, Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland

Received August 18, 1998; accepted March 7, 1999

This paper is available online at http://www.molpharm.org

ABSTRACT

A variety of direct and indirect techniques have revealed the existence of ATP-sensitive potassium (K<sub>ATP</sub>) channels in the inner membranes of mitochondria. The molecular identity of these mitochondrial K<sub>ATP</sub> (mitoK<sub>ATP</sub>) channels remains unclear. We used a pharmacological approach to distinguish mitoK<sub>ATP</sub> channels from classical, molecularly defined cardiac sarcolemmal K<sub>ATP</sub> (surfaceK<sub>ATP</sub>) channels encoded by the sulfonylurea receptor SUR2A and the pore-forming subunit Kir6.2. SUR2A and Kir6.2 were expressed in human embryonic kidney (HEK) cells and their activities were measured by patch-clamp recordings of membrane current. SurfaceK<sub>ATP</sub> channels are activated potently by 100 μM pinacidil but only weakly by 100 μM diazoxide; in addition, they are blocked by 10 μM glibenclamide, but are insensitive to 500 μM 5-hydroxydecanoate. This pharmacology, which was confirmed with patch-clamp recordings in intact rabbit ventricular myocytes, contrasts with that of mitoK<sub>ATP</sub> channels as indexed by flavoprotein oxidation. MitoK<sub>ATP</sub> channels in myocytes are activated equally by 100 μM diazoxide and 100 μM pinacidil. In contrast to its lack of effect on surfaceK<sub>ATP</sub> channels, 5-hydroxydecanoate is an effective blocker of mitoK<sub>ATP</sub> channels. Glibenclamide’s effects on mitoK<sub>ATP</sub> channels are difficult to assess, because its inhibition is independent of the sulfonylurea receptor SUR2A. It is possible that it acts directly on the Kir6.2 pore-forming subunits, and that its effects on mitoK<sub>ATP</sub> channels are not significant. Moreover, surfaceK<sub>ATP</sub> channels are activated by pinacidil and inhibited by glibenclamide, whereas mitoK<sub>ATP</sub> channels are activated by pinacidil and inhibited by glibenclamide. Native cardiac mitoK<sub>ATP</sub> channels share some pharmacological properties with their classical, sarcolemmal counterparts. Both channels are activated by pinacidil and inhibited by glibenclamide (Paucek et al., 1992; Garlid et al., 1997). Thus, specific inhibitors and activators are in demand for the study of cardiac mitoK<sub>ATP</sub> channels. 5-hydroxydecanoate (SHD) and diazoxide are candidates for such specific reagents (Garlid et al., 1997; Liu et al., 1998). Nevertheless, there is controversy as to whether SHD blocks surfaceK<sub>ATP</sub> channels (Notsu et al., 1992; Garlid et al., 1997; Liu et al., 1998). Likewise, the extent to which the activator diazoxide opens surfaceK<sub>ATP</sub> channels is a matter of some dispute (Inagaki et al., 1996; Garlid et al., 1997; Liu et al., 1998).

In 1986, Murry and coworkers (Murry et al., 1986) described a paradoxical form of cardioprotection whereby brief ischemic insults can reduce the damage to the heart caused by subsequent, prolonged ischemia. This phenomenon, known as ischemic preconditioning, appears to involve ATP-sensitive potassium (K<sub>ATP</sub>) channels as the end effectors. Such a key role was first attributed to the classical sarcolemmal K<sub>ATP</sub> (surfaceK<sub>ATP</sub>) channels (Gross and Auchampach, 1992; Speichly-Dick et al., 1995; Liang, 1997; Kloner et al., 1992), and recent evidence implicates the more nebulous mitochondrial K<sub>ATP</sub> (mitoK<sub>ATP</sub>) channels (Inoue et al., 1991; Garlid et al., 1997; Liu et al., 1998; Sato et al., 1998). The interplay between these two K<sub>ATP</sub> channel populations is at the heart of many of the questions surrounding the role of K<sub>ATP</sub> channels in the myocardium.

Native cardiac mitoK<sub>ATP</sub> channels share some pharmacological properties with their classical, sarcolemmal counterparts. Both channels are activated by pinacidil and inhibited by glibenclamide (Paucek et al., 1992; Garlid et al., 1997). Thus, specific inhibitors and activators are in demand for the study of cardiac mitoK<sub>ATP</sub> channels. 5-hydroxydecanoate (SHD) and diazoxide are candidates for such specific reagents (Garlid et al., 1997; Liu et al., 1998). Nevertheless, there is controversy as to whether SHD blocks surfaceK<sub>ATP</sub> channels (Notsu et al., 1992; Garlid et al., 1997; Liu et al., 1998). Likewise, the extent to which the activator diazoxide opens surfaceK<sub>ATP</sub> channels is a matter of some dispute (Inagaki et al., 1996; Garlid et al., 1997; Liu et al., 1998). The molecular identity of cardiac mitoK<sub>ATP</sub> channels remains unclear, although cardiac surfaceK<sub>ATP</sub> channels have been molecularly defined as an octameric complex of four pore-forming Kir<sub>6.2</sub> subunits and four SUR2A sulfonylurea receptors (Inagaki et al., 1996; Clement et al., 1997). To

ABBREVIATIONS: SHD, 5-hydroxydecanoate; DNP, 2,4-dinitrophenol; GFP, green fluorescent protein; EGFP, enhanced GFP; HEK, human embryonic kidney; K<sub>ir</sub>, inwardly rectifying potassium channels; K<sub>ATP</sub> channels, ATP-sensitive potassium channels; mitoK<sub>ATP</sub>, inner mitochondrial membrane K<sub>ATP</sub>; SUR, Sulfonylurea receptor; surfaceK<sub>ATP</sub>, sarcolemmal (surface membrane) K<sub>ATP</sub>.
distinguish unambiguously between surface\(K_{\text{ATP}}\) and mito\(K_{\text{ATP}}\) channels, we compared native cardiac mito\(K_{\text{ATP}}\) channels to molecularly defined and native surface\(K_{\text{ATP}}\) channels. Differences in pharmacology and subcellular distribution reveal clear distinctions between cardiac mito\(K_{\text{ATP}}\) and surface\(K_{\text{ATP}}\) channels.

Materials and Methods

Functional Expression of \(K_{\text{ir6.2}} + \text{SUR2A Channels.}\) The transient transfection method in human embryonic kidney (HEK/293 cells was modified from Hu et al. (1997). Briefly, HEK293 cells were maintained in Dulbecco’s minimal Eagle’s medium with glucose and L-glutamine, supplemented with 10% fetal calf serum (GIBCO-BRL, Gaithersburg, MD) and 1% penicillin and streptomycin (GIBCO-BRL). Cells were plated on 35-mm Petri dishes at a density of 0.2 million cells/dish 1 day before transfection, and maintained in a 37°C incubator. Cells were then transfected by calcium phosphate precipitation (Graham and van der Eb, 1973; Calcium Phosphate Transfection System, GIBCO-BRL) with 0.5 to 1 \(\mu\)g/dish of equimolar plasmid DNAs of the rabbit cardiac \(K_{\text{ir6.2}}\) subunit (Hu et al., 1999) and the rat SUR2A sulfonylurea receptor (Inagaki et al., 1996), supplemented with 0.2 \(\mu\)g/dish of mitochondrially targeted green fluorescent protein, which enabled visual identification of transfected cells (Marshall et al., 1995). The calcium phosphate-DNA mixture was left on cells for 5 to 6 h before washing with PBS and the addition of fresh media.

Electrophysiology of Expressed \(K_{\text{ir6.2}} + \text{SUR2A Channels.}\) For functionally expressed surface\(K_{\text{ATP}}\) channels, electrophysiologic recordings were made 18 to 48 h after transfection. A coverslip with cells was placed in a 0.3-ml perfusion chamber connected to a gravity-driven perfusion system. Flow was maintained throughout the experiment at rates of 2 to 3 ml/min. Membrane current was recorded using the whole-cell patch configuration (Hamill et al., 1981), with bath solution containing (in mM): 140 NaCl, 5 KCl, 1 CaCl\(_2\), 1 MgCl\(_2\), and 10 HEPES, pH 7.4 titrated with NaOH. The pipette solution contained: 120 mM K-glutamate, 5 mM K-EGTA, 25 mM KCl, 3 mM MgATP, and 10 mM HEPES, pH 7.2, titrated with KOH (21–22°C). Junction potential was corrected. In voltage-clamp recordings, a ramp voltage command from \(-100\) mV to \(+50\) mV was applied over 100 ms every 10 s, from a holding potential of \(-80\) mV. Currents were recorded using a patch-clamp amplifier (Axopatch 200, Axon Instruments, Inc.), and sampled at 10 kHz after analog filtering at 2 to 5 kHz. Acquisition and analysis of the data were performed with custom software. For data analysis, the current at 0 mV was measured to assay \(K_{\text{ATP}}\) channel activity. The holding current at \(-80\) mV (near \(E_{\text{K}}\)) was also measured to verify the gigaseal stability. In the study of the effect of 5HD on membrane current, 1 min of \(K_{\text{ATP}}\) currents before drug application were averaged as the control, and the currents 5 to 6 min into drug application were averaged for comparison with the control. The effect of reagent diazoxide was assayed in a similar way, except that steady-state currents (typically achieved within 5–10 min) were measured.

Confocal Imaging Green Fluorescent Protein (GFP)-\(K_{\text{ir6.2}}\), Mitochondrial GFP, and Mitochondria. The murine \(K_{\text{ir6.2}}\) cDNA (Inagaki et al., 1995) was amplified by the polymerase chain reaction and cloned into the vector pEGFP-C3 (CLONTECH, Palo Alto, CA) to make pEGFP-Kir6.2. This created a single open reading frame encoding a fusion protein between EGFP on the amino terminus and Kir6.2 on the carboxy terminus. Stable cell lines were created by transfecting HEK293 cells with linearized pEGFP-Kir6.2 using lipofectamine (Life Technologies, Gaithersburg, MD) followed by selection with genetinic sulfate at 500 \(\mu\)g/ml. Cells surviving selection were plated on glass coverslips and imaged. Mitochondrially targeted GFP (Rizzuto et al., 1995) was transiently transfected into HEK293 cells as described above, and the cells were imaged. Terminally differentiated muscle cells such as heart cells are notoriously resistant to conventional transfection methods. To image the subcellular distribution of fluorescent Kir6.2 in cardiac myocytes, we created adenoviral vectors for infection of rabbit ventricular cells (isolated and cultured as described below). We first generated an isogeneic Kir6.2.GFP fusion construct by amplifying the coding region of the rabbit Kir6.2 gene (Hu et al., 1999) by polymerase chain reaction to allow for in-frame fusion with EGFP in the vector pEGFP-N3 (CLONTECH) to create pHabKir6.2.GFP. The expression cassette was removed and cloned into the multiple cloning site of modified adenovirus shuttle vector pAdECD8I (Johns et al., 1999) to generate pAdECD8Kir6.2.GFP. The recombinant adenovirus containing the RabKir6.2.GFP fusion gene product was generated by Cre-lox recombination as described previously (Johns et al., 1999). Isolated rabbit ventricular myocytes were infected with AdRabKir6.2.GFP and AdVGKXR (Johns et al., 1999) and plated on to coverslips in M199 culture medium with 5% fetal bovine serum at 37°C. Expression was induced by adding 5 \(\mu\)M ponasterone A for 72 h before imaging.

Confocal images were obtained using a 60× water immersion lens on a Diaphot 300 inverted fluorescence microscope with a PCM-2000 confocal scanning attachment (Nikon, Inc., Melville, NY). GFP was excited by the 488-nm line of an argon laser and the emission at 505 to 535 nm was imaged. Mitochondria were stained with tetramethylrhodamine ethyl ester (TMRE, added to the bath at a concentration of 100 nM and allowed to redistribute into the mitochondrial matrix for 15 min). TMRE was excited with the 543-nm line of a helium-neon laser and the 589- to 621-nm emission was imaged.

Flavoprotein Fluorescence and Electrophysiology of Rabbit Ventricular Myocytes. Ventricular myocytes were isolated from adult rabbit hearts by conventional enzymatic dissociation (Liu et al., 1996). Cells were then cultured on laminin-coated coverslips in M199 culture medium with 5% fetal bovine serum at 37°C. Experiments were performed over the next 2 days. Mitochondrial redox state was monitored by recording the fluorescence of flavin adenine nucleotide (FAD)-linked enzymes in the mitochondria and served as an index of mito\(K_{\text{ATP}}\) activity (Liu et al., 1998). Myocytes were superfused with external solution containing: 140 mM NaCl, 5 mM KCl, 1 mM CaCl\(_2\), 1 mM MgCl\(_2\), and 10 mM HEPES, pH 7.4, with NaOH (21–22°C). Endogenous flavoprotein fluorescence was excited for 100 ms every 6 s using a xenon arc lamp with a band-pass filter centered at 480 nm, and emitted fluorescence was recorded at 530 nm by a photomultiplier tube and digitized (Digidata 1200, Axon Instruments, Foster City, CA). By focusing on individual myocytes with a 40× objective, fluorescence was monitored from one cell at a time. The flavoprotein fluorescence signal was averaged during the excitation window and expressed as a percentage of the 2,4-dinitrophenol (DNP)-induced maximal oxidation.

In some experiments, whole-cell currents were recorded from myocytes. The internal pipette solution contained: 120 mM K-glutamate, 25 mM KCl, 0.5 mM MgCl\(_2\), 10 mM K-EGTA, 10 mM HEPES, and 1 mM MgATP (pH 7.2 with KOH). Whole-cell currents were elicited every 6 s from a holding potential of \(-80\) mV by two consecutive steps to \(-40\) mV (for 100 ms) and 0 mV (for 380 ms). Currents at 0 mV were measured 200 ms into the pulse.

Chemicals. Diazoxide and glibenclamide were purchased from Sigma Chemical Co. (St. Louis, MO). Pinacidil and 5HD were purchased from Research Biochemical International (Natick, MA). TMRE was obtained from Molecular Probes (Eugene, OR). To prepare stock solutions, 5HD and DNP were dissolved in water, and the other drugs were dissolved in dimethyl sulfoxide. Ponasterone A was purchased from Invitrogen (San Diego, CA) and used according to the manufacturer’s instructions.

Statistical Analyses. Pooled data are presented as mean ± S.E.M. Statistical comparison was evaluated by the two-tailed paired or unpaired Student’s \(t\) test where appropriate, with \(P < .05\) considered significant.
Results

5HD Has Little Effect on Expressed Cardiac SurfaceK<sub>ATP</sub> Channels. Figure 1 shows the effect of 5HD on currents elicited by 100 μM pinacidil in a HEK293 cell expressing K<sub>ir6.2</sub> + SUR2A, which forms cardiac-type surfaceK<sub>ATP</sub> channels (Inagaki et al., 1996). Figure 1A shows a time course of pharmacological responses in a representative experiment quantified from membrane currents elicited by ramp pulses (as exemplified in Fig. 1B); these data illustrate that 500 μM 5HD did not convincingly inhibit the current, but subsequent application of glibenclamide (10 μM) did so completely. Fig. 1C shows the pooled results in a box graph, presented as the ratio of the current in the presence of both 5HD and pinacidil, to that originally induced by pinacidil alone. Overall, 5HD imposed no significant inhibitory effect (3 ± 3%, n = 5).

Diazoxide Weakly Activates Expressed Cardiac SurfaceK<sub>ATP</sub> Currents. Diazoxide weakly activated expressed K<sub>ATP</sub> currents, as illustrated in Fig. 2. Upon washout of diazoxide, a subsequent application of 100 μM pinacidil activated much more current. Overall, 100 μM diazoxide elicited only 4 ± 1% (n = 26, P < 0.03) of the current activated by 100 μM pinacidil, which is a nearly saturating concentration in this expression system (Hu et al., 1998). Diazoxide-induced currents were completely suppressed by glibenclamide (n = 3, not shown).

Given that 100 μM diazoxide activates mitoK<sub>ATP</sub>, but not surfaceK<sub>ATP</sub> channels in native cardiomyocytes (Garlid et al., 1997; Liu et al., 1998), we questioned whether the minor activation of the expressed channels might have actually reflected cross talk between the mitochondrial and the sarcoplasmic reticulum. We thus examined the effect of 5HD on diazoxide-induced currents in the expression system. Figure 2 shows the effect of 100 μM diazoxide and 500 μM 5HD on
cells expressing K<sub>ir6.2</sub> + SUR2A channels. Diazoxide reversibly activated a small outward current at 0 mV. In the presence of 5HD, a second challenge with diazoxide induced a current comparable with the first one. After washing out diazoxide and 5HD, pinacidil activated a robust current, verifying that the channels were richly expressed. The inset shows pooled data of the effect of 5HD, presented as the ratio of the second current (diazoxide + 5HD) versus the first one (diazoxide only). Overall, 5HD had no effect on diazoxide-induced currents. Thus, diazoxide weakly activates surfaceK<sub>ATP</sub> channels encoded by K<sub>ir6.2</sub> and SUR2A.

Surface Membrane K<sub>ATP</sub> Currents in Heart Cells. The pattern of pharmacologic responsiveness observed for heterologously expressed channels encoded by K<sub>ir6.2</sub> and SUR2A also applies to the native channels in rabbit cardiac myocytes. We have previously shown that such channels are resistant to diazoxide but sensitive to pinacidil (Liu et al., 1998; Sato et al., 1998). Figure 3 shows that pinacidil readily elicits outward membrane current in a rabbit ventricular cell, an effect that cannot be suppressed by 5HD. The pooled data (Fig. 3B) extend our previous observations (Sato et al., 1998); taken together, the data in Figs. 1–3 verify that the molecularly defined surfaceK<sub>ATP</sub> channels mimic the behavior of native cardiac surfaceK<sub>ATP</sub> channels.

K<sub>ir6.2</sub> Does Not Target Mitochondrial Membranes. To address whether expressed K<sub>ir6.2</sub> subunits are targeted to mitochondrial membranes, we imaged HEK293 cells stably expressing the fusion construct GFP-K<sub>ir6.2</sub>. Figure 4A–C shows the distribution of expressed K<sub>ir6.2</sub> subunits (Fig. 4A, green), the mitochondria of the cells (Fig. 4B, labeled red by TMRE), and the overlay image (Fig. 4C). Green signals are distributed in both the surface and internal membranes of cells, but this distribution does not overlap that of the red mitochondria. As a positive control, we imaged HEK293 cells transiently expressing mitochondrially targeted GFP in the same manner. The GFP shows up in the same organelles (Fig. 4D, green) that are labeled red by TMRE (Fig. 4E), making them yellow in the overlay image (Fig. 4F).

Expression of isogeneic fluorescently labeled Kir6.2 in cardiac myocytes revealed a similar lack of concordance between the subcellular distribution of the channel (Fig. 5A) and the

Fig. 1. Effects of 5HD on pinacidil-induced surfaceK<sub>ATP</sub> channels expressed in HEK293 cells. A, time course of the membrane current measured at 0 mV (○) in the presence or absence of 100 μM pinacidil (Pin), 500 μM 5HD, or 10 μM glibenclamide (Glib). The holding current at −80 mV is also plotted (■). B, representative ramp currents recorded under drug-free conditions (a), when pinacidil was applied (b), when 5HD was added (c), and when glibenclamide was added (d), at the times indicated in A. Pinacidil was present (b–d). C, pooled data from five experiments of the relative current I<sub>5HD+Pin</sub> versus I<sub>Pin</sub>, i.e., the current at time c versus that at time b as indicated in A.

Fig. 2. Effects of diazoxide and 5HD on K<sub>ir6.2</sub> + SUR2A channels expressed in a HEK293 cell. The cell was first exposed to 100 μM diazoxide (Diaz) alone, then again in the presence of 500 μM 5HD, and finally to 100 μM pinacidil (Pin). The inset box graph shows the effect of 5HD from seven experiments expressed as the ratio of the second current elicited by diazoxide + 5HD versus the first current with diazoxide alone (P = N.S.).
mitochondria (Fig. 5B). From these experiments, we conclude that Kir6.2 subunits do not target mitochondrial membranes.

**5HD Inhibits mitoK\textsubscript{ATP} in Intact Rabbit Ventricular Myocytes.** Having studied the effects of diazoxide and 5HD on expressed surfaceK\textsubscript{ATP} channels, we then examined their effects on the native mitoK\textsubscript{ATP} in rabbit cardiomyocytes. We previously established single-cell methodologies to assay surfaceK\textsubscript{ATP} and mitoK\textsubscript{ATP} channel activities by measuring membrane current and flavoprotein fluorescence simultaneously (Liu et al., 1998; Sato et al., 1998); in the present study, we verified the effects of the reagents on mitoK\textsubscript{ATP} channels in intact rabbit ventricular myocytes not invaded by patch pipettes.

Figure 6 shows that diazoxide (100 \( \mu \)M) reversibly increased the flavoprotein oxidation, and the oxidative effect was inhibited by 500 \( \mu \)M 5HD. DNP was introduced at the mitochondria (Fig. 5B). From these experiments, we conclude that Kir6.2 subunits do not target mitochondrial membranes.

![Figure 3](image3.png)

**Fig. 3.** Pharmacological sensitivity of native surfaceK\textsubscript{ATP} channels in rabbit ventricular myocytes. A, time course of membrane current elicited by pinacidil (100 \( \mu \)M) in the absence and presence of 5HD (500 \( \mu \)M). B, pooled data for pinacidil-induced membrane current measured in the absence and presence of 5HD. \( P \) = N.S.

![Figure 4](image4.png)

**Fig. 4.** Confocal fluorescent images of HEK293 cells stably expressing Kir6.2 fused with GFP (left column, A–C), and HEK293 cells transiently expressing mitochondrial GFP (right column, D–F). Green signals indicate the locations of GFP-Kir6.2 (A) and mitochondrially targeted GFP (D), and red signals reveal the locations of mitochondria as the cells were loaded with TMRE (B and E). C and F, overlay images of A and B, and D and E, respectively.
end of each experiment to determine the maximal level of oxidation. As summarized in Fig. 6B, diazoxide increased flavoprotein oxidation to 38 ± 6% of that induced by DNP (n = 5), a value equivalent to the 34 ± 4% induced by 100 μM pinacidil (Sato et al., 1998). 5HD inhibited the effect of diazoxide to 7 ± 2% of the DNP level (n = 5, P < .005 versus diazoxide alone). These results indicate that diazoxide activates, and 5HD inhibits, mitoK<sub>ATP</sub> channels in intact rabbit ventricular myocytes.

**Glibenclamide Independently Activates Flavoprotein Fluorescence in Intact Rabbit Cardiomyocytes.** Glibenclamide, a well-known sulfonylurea K<sub>ATP</sub> channel blocker, has also been reported to be an uncoupler of mitochondria (Szewczyk et al., 1997). On uncoupling respiration from ATP synthesis, the mitochondrial redox potential is oxidized like that induced by DNP. We therefore examined the effect of glibenclamide on flavoprotein fluorescence. Figure 7 illustrates one such experiment. Glibenclamide (100 μM) induced reversible oxidation of the flavoprotein. This effect was concentration dependent. As summarized in Fig. 7B, 10 and 100 μM glibenclamide increased mitochondrial oxidation to 15 ± 4% (n = 4) and 27 ± 4% (n = 5), respectively. Thus, glibenclamide is not a useful probe of mitoK<sub>ATP</sub>-induced mitochondrial oxidation.

**Discussion**

We have shown that expressed cardiac K<sub>ATP</sub> channels (Kir6.2 + SUR2A) are activated strongly by pinacidil but only weakly by diazoxide, while being blocked by glibenclamide and essentially insensitive to 5HD. This pharmacology contrasts with that of mitoK<sub>ATP</sub> channels as indexed by flavoprotein oxidation in intact rabbit ventricular myocytes. MitoK<sub>ATP</sub> channels are activated equally by diazoxide and pinacidil, and 5HD is an effective blocker of mitoK<sub>ATP</sub> channels. These differences establish major pharmacological distinctions between the two channels at a functional level. In addition, confocal imaging reveals that expressed Kir6.2 subunits target sarcolemmal but not mitochondrial membranes. These key distinctions indicate that mitoK<sub>ATP</sub> channels differ importantly from surfaceK<sub>ATP</sub> channels at the molecular level.

In this study, we provide the first direct evidence that glibenclamide alone (10 μM or 100 μM) causes mitochondrial oxidation. This effect of glibenclamide precludes investigation in intact cells of its reported inhibition of mitoK<sub>ATP</sub> channels in isolated mitochondria or reconstituted liposomes (Inoue et al., 1991; Paucek et al., 1992); nevertheless, the observations are consistent with the reported uncoupling effect of glibenclamide on mitochondria (Szewczyk et al., 1997). In addition, glibenclamide effectively blocks both native and expressed cardiac surfaceK<sub>ATP</sub> channels (Edwards and Weston, 1993; Inagaki et al., 1996; Hu et al., 1999). Thus, glibenclamide is a less-than-optimal probe in the study of mitoK<sub>ATP</sub> channels.

The results with glibenclamide illustrate one limitation of the methodology that we have used to detect mitoK<sub>ATP</sub> activity. The method relies upon flavoprotein fluorescence to...
report the redox potential of the mitochondrial matrix. Although opening of inner membrane potassium channels will tend to dissipate the mitochondrial potential and lead to net oxidation of the matrix, so will uncouplers such as glibenclamide and DNP (Liu et al., 1998). The only other approaches devised so far to detect mitoKATP activity use isolated mitochondria (Inoue et al., 1991; Garlid et al., 1997), which necessarily involves removing the organelles from their physiological surroundings. Thus, the two types of approaches have complementary strengths and limitations. It is therefore reassuring that similar conclusions regarding the pharmacology of mitoKATP channels have been derived from studies of flavoprotein fluorescence in intact myocytes and potassium accumulation in isolated mitochondria (Garlid et al., 1997; Liu et al., 1998).

Identifying a specific mitoKATP channel blocker is very important, especially given that mitoKATP channels have been pinpointed as likely effectors of ischemic preconditioning (Garlid et al., 1997; Liu et al., 1998). Our data identify 5HD as a specific blocker of mitoKATP channels. At 500 μM, 5HD did not inhibit pinacidil-induced currents through expressed or native cardiac surfaceKATP channels, nor did it suppress diazoxide-induced surfaceKATP currents. In native cardiomyocytes, 5HD dramatically suppresses mitoKATP channel activity, yet it does not affect surfaceKATP currents (McCullough et al., 1991; Garlid et al., 1997; Liu et al., 1998; Sato et al., 1998), with the exception of one report that 5HD blocks surfaceKATP channels in excised patches from guinea pig cardiomyocytes (Notsu et al., 1992). The reason for this contradictory observation remains unclear. In animal studies, 5HD has long been recognized to abolish cardioprotection (Auchampach et al., 1992; Hide and Thiemermann, 1996; Schultz et al., 1997). The establishment of its specificity further implicates mitoKATP channels as key players in the mechanism of ischemic preconditioning.

Acknowledgments

We thank Ms. Maria Janecki for technical assistance, Dr. Susumu Seino for providing SUR2A, and Dr. Yoshihisa Kurachi for providing mouse K_6.2.

References


Send reprint requests to: Eduardo Marbán, M.D., Ph.D., 844 Ross Bldg., The Johns Hopkins University School of Medicine, Baltimore, MD 21205. E-mail: marban@jhmi.edu

Copyright © The American Society for Pharmacology and Experimental Therapeutics. All rights reserved.