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# Pharmacological Comparison of Native Mitochondrial $K_{ATP}$ Channels with Molecularly Defined Surface $K_{ATP}$ Channels

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## ABSTRACT

Many mammalian cells have two distinct types of ATP-sensitive potassium ($K_{ATP}$) channels: the classic ones in the surface membrane ($sK_{ATP}$) and others in the mitochondrial inner membrane ($mitoK_{ATP}$). Cardiac $mitoK_{ATP}$ channels play a pivotal role in ischemic preconditioning, and thus represent interesting drug targets. Unfortunately, the molecular structure of $mitoK_{ATP}$ channels is unknown, in contrast to $sK_{ATP}$ channels, which are composed of a pore-forming subunit (Kir6.1 or Kir6.2) and a sulfonylurea receptor (SUR1, SUR2A, or SUR2B). As a means of probing the molecular makeup of $mitoK_{ATP}$ channels, we compared the pharmacology of native cardiac $mitoK_{ATP}$ channels with that of molecularly defined $sK_{ATP}$ channels expressed heterologously in human embryonic kidney 293 cells. Using mitochondrial oxidation to index $mitoK_{ATP}$ channel activity in rabbit ventricular myocytes, we found that pinacidil and diazoxide open $mitoK_{ATP}$ channels, but P-1075 does not. On the other hand, 5-hydroxydecanoic acid (5HD), but not HMR-1098, blocks $mitoK_{ATP}$ channels. Although pinacidil is a nonselective activator of expressed $sK_{ATP}$ channels, diazoxide did not open channels formed by Kir6.1/SUR2A, Kir6.2/SUR2A (known components of cardiac $sK_{ATP}$ channels) or Kir6.2/SUR2B. P-1075 activated all the $K_{ATP}$ channels, except Kir6.1/SUR1 channels. Glybenclamide potently blocked all $sK_{ATP}$ channels, but 5HD only blocked channels formed by SUR1/Kir6.1 or Kir6.2 ($IC_{50}$s of 66 and 81 $\mu$M, respectively). This potency is similar to that for block of $mitoK_{ATP}$ channels ($IC_{50}$ = 95 $\mu$M). In addition, HMR-1098 potently blocked Kir6.2/SUR2A channels ($IC_{50}$ = 1.5 $\mu$M), but was 67 times less potent in blocking Kir6.1/SUR1 channels ($IC_{50}$ = 100 $\mu$M). Our results demonstrate that $mitoK_{ATP}$ channels closely resemble Kir6.1/SUR1 $sK_{ATP}$ channels in their pharmacological profiles.

Lethal injury to the heart can be dramatically blunted by brief conditioning periods of ischemia. Such "ischemic preconditioning" (IPC) (Murry et al., 1986) exists in all species examined, including humans (Cohen and Downey, 1993). Although the precise mechanism of IPC remains elusive, much attention has focused on the potential role of ATP-sensitive potassium ($K_{ATP}$) channels as the effectors of protection. Cardiac myocytes contain two distinct $K_{ATP}$ channels: the classic one in the sarcolemma (Noma, 1983) and another in the mitochondrial inner membrane ($mitoK_{ATP}$ channel) (Inoue et al., 1991). Although the cardioprotection was originally attributed to sarcolemmal $K_{ATP}$ channels, recent evidence has pinpointed $mitoK_{ATP}$ channels as the key effectors of cardioprotection (Garlid et al., 1997; Liu et al., 1998, 1999).

Molecular studies have revealed that surface membrane $K_{ATP}$ ($sK_{ATP}$) channels are octameric complexes of four pore-forming Kir6.x subunits and four sulfonylurea subunits (Aguilar-Bryan et al., 1998). Two isoforms of Kir (Kir6.1 and Kir6.2) and three of SUR (SUR1, SUR2A, and SUR2B) have been identified. $sK_{ATP}$ channels are broadly distributed but quite tissue-specific in their expression patterns. For example, Kir6.2/SUR1 forms the pancreatic $\beta$-cell $sK_{ATP}$ channel, whereas Kir6.2/SUR2A is the cardiac $sK_{ATP}$ channel (Yokoshiki et al., 1998). Kir6.1/SUR2B and Kir6.2/SUR2B form vascular smooth muscle $sK_{ATP}$ channels (Isomoto et al., 1996; Yamada et al., 1997) and various permutations have been reported in neuronal cells (Miller et al., 1999). However, the molecular structure of the $mitoK_{ATP}$ channel has not been determined. In this study, we compared the pharmacological profiles of the native $mitoK_{ATP}$ channels in rabbit ventricular myocytes with heterologously expressed $K_{ATP}$ channels in HEK293 cells. Mitochondrial oxidation was used as an indirect index of $mitoK_{ATP}$ channel opening in myocytes (Liu et al., 1998). All possible combinations of $sK_{ATP}$ subunits (Kir6.1/SUR1, Kir6.1/SUR2A, Kir6.1/SUR2B, Kir6.2/SUR1, Kir6.2/SUR2A, and Kir6.2/SUR2B) were heterologously ex-

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**ABBREVIATIONS:** IPC, ischemic preconditioning; $K_{ATP}$, ATP-sensitive potassium channel; $mitoK_{ATP}$, mitochondrial ATP-sensitive potassium channel; $sK_{ATP}$, surface membrane ATP-sensitive potassium channel; Kir, inward rectifying potassium channel; SUR, sulfonylurea receptor; HEK, human embryonic kidney; 5HD, 5-hydroxydecanoic acid sodium.

pressed in HEK293 cells, and their pharmacology was characterized with the whole-cell, patch-clamp technique. Comparison of the results reveals striking similarities between the pharmacological profiles of Kir6.1/SUR1 and mito$K_{ATP}$ channels.

## Materials and Methods

The investigation conforms with *The Guide for the Care and Use of Laboratory Animals*, published by the National Research Council in 1996 and approved by the Institutional Animal Care and Use Committee.

**Chemicals.** Collagenase (type II) was purchased from Worthington (Freehold, NJ). Diazoxide was obtained from Sigma Chemical Co. (St. Louis, MO). Pinacidil and 5-hydroxydecanoic acid sodium (5HD) were purchased from Research Biochemical International (Natick, MA). HMR-1098 was a gift from Aventis Pharma (Frankfurt, Germany) and P-1075 was a gift from Leo Pharmaceutical Products (Ballerup, Denmark). Diazoxide, pinacidil, and P-1075 were dissolved in dimethyl sulfoxide before being added into experimental solutions. The final concentration of dimethyl sulfoxide was $< 0.1\%$.

**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. For whole-cell patch recordings, 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. The external solution included 140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES, pH 7.4 with NaOH. 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. Endogenous flavoprotein fluorescence was excited using a xenon arc lamp with a bandpass filter centered at 480 nm, but only during the 100-ms step to −40 mV to minimize photobleaching. Emitted fluorescence was recorded at 530 nm by a photomultiplier tube and digitized (Digidata 1200; Axon Instruments, Foster City, CA). Relative fluorescence was averaged during the excitation window and calibrated using the values after dinitrophenol and sodium cyanide exposure (Liu et al., 1998).

**Functional Expression of $K_{ATP}$ Channels and Electrophysiology.** Human embryonic kidney cells were plated at a density of $1 \times 10^5$ cells per 35-mm dish with glass coverslips in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Plasmid DNA (3 $\mu$g) containing both the Kir (6.1 or 6.2) and SUR (1, 2A, or 2B) cDNA was transfected using Lipofectamine Plus (Life Technologies, Gaithersburg, MD) 18 h after splitting the cells. Mouse Kir6.1 [a kind gift of Dr. Y. Kurachi (Yamada et al., 1997)] and rabbit Kir6.2 (Hu et al., 1999) were cloned into vector pGFP-IRES (Johns et al., 1997). Hamster SUR1 [a kind gift from Dr. Bryan (Aguilar-Bryan et al., 1995)] was cloned into expression vector pCDNA3.1 (Invitrogen). Rat SUR2A [kindly provided by Dr. Seino (Inagaki et al., 1996)] was cloned into mammalian expression vector pCMV6. Mouse SUR2B was cloned into expression vector pCDNA3 and was a kind gift of Dr. Kurachi (Yamada et al., 1997).

Whole-cell electrophysiology recordings were made 48 to 72 h after transfection with solutions identical to those used in rabbit ventricular myocytes (see above) except that in the pipette solution, 3 mM MgATP was used. Voltage ramps from −100 mV to +50 mV were applied over 400 ms every 2 s, from a holding potential of −20 mV. The currents at 0 mV and −70 mV were measured to assay $K_{ATP}$ channel activity and seal stability, respectively. Experiments were performed at room temperature (≈22°C).

Three $K_{ATP}$ channel openers (diazoxide, pinacidil, and P-1075) and three $K_{ATP}$ channel blockers (glybenclamide, 5HD, and HMR-1098) were used to probe the pharmacological characteristics of six (Kir6.1/SUR1, Kir6.1/SUR2A, Kir6.1/SUR2B, Kir6.2/SUR1, Kir6.2/SUR2A, and Kir6.2/SUR2B) heterologously expressed $K_{ATP}$ channels and native mito$K_{ATP}$ channels in rabbit ventricular myocytes. The pharmacological profiles for each opener or blocker of the six heterologously expressed $K_{ATP}$ channels were then compared with those of native mito$K_{ATP}$ channels. The data are presented as mean ± S.E.M.

## Results

**Pharmacology of Heterologously Expressed $K_{ATP}$ Channels.** Fig. 1 summarizes the $K_{ATP}$ current densities (measured at 0 mV during the ramp) activated by diazoxide (100 $\mu$M), pinacidil (100 $\mu$M), or P-1075 (100 $\mu$M) and after glybenclamide (10 $\mu$M), 5HD (200 $\mu$M), and HMR-1098 (10 $\mu$M). All combinations of Kir6.x and SUR were opened by 100 $\mu$M pinacidil and blocked by 10 $\mu$M glybenclamide. Kir6.1 combinations with SUR1 or 2B were opened by diazoxide; of the Kir6.2 combinations, however, only the Kir6.2/SUR1 ($\beta$-cell type s$K_{ATP}$ channels) was sensitive to this compound. Blockade by 5HD was associated with SUR1 expression partnered with either Kir subunit, whereas blockade by HMR-1098 seemed to be selective for Kir6.2 coexpressed with either SUR1 or SUR2A (cardiac s$K_{ATP}$ channels), but not SUR2B [vascular $K_{ATP}$ channels (Yamada et al., 1997)]. P-1075 (100 $\mu$M) activated all the $K_{ATP}$ channels, except the construct with Kir6.1/SUR1.

Figure 2 shows the time course of a typical experiment on Kir6.1/SUR1 during the application of various drugs. The channels clearly and reproducibly respond to diazoxide (but not to P-1075), and the diazoxide response can be blocked by 5HD. As considered below, this pattern of responsiveness parallels that described for mito$K_{ATP}$ channels.

**Pharmacological Comparison of mito$K_{ATP}$ Channels to Heterologously Expressed $K_{ATP}$ Channels.** Table 1 compares the pharmacology of heterologously expressed $K_{ATP}$ channels from this study with the previously-described responses of mito$K_{ATP}$ channels to the same openers and

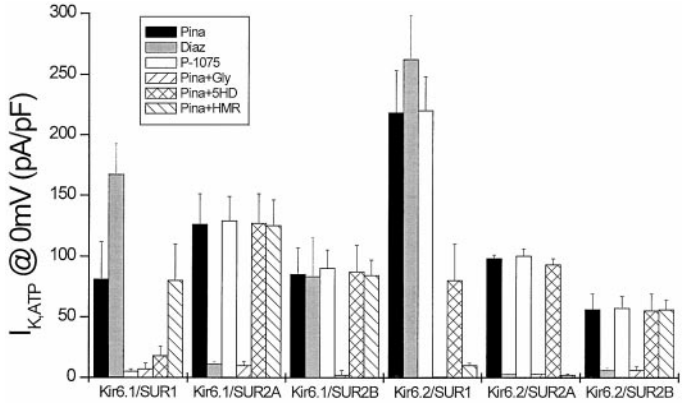


**Fig. 1.** A summary of $K_{ATP}$ currents measured at 0 mV for all six types of $K_{ATP}$ channel heterologously expressed in HEK293 cells. Pina (100 $\mu$M pinacidil) activated, and Gly (10 $\mu$M glybenclamide) blocked all six types of $K_{ATP}$ channels. Dia (100 $\mu$M diazoxide) did not activate the channels formed by Kir6.2/SUR2A or Kir6.2/SUR2B. P-1075 (100 $\mu$M) activated all the heterologously expressed $K_{ATP}$ channels, except Kir6.1/SUR1. 5HD (200 $\mu$M) blocked only the channels formed by Kir6.1/SUR1 and Kir6.2/SUR1. On the other hand, HMR (10 $\mu$M HMR-1098) had no blocking effect on Kir6.1/SUR1, Kir6.1/SUR2A, Kir6.1/SUR2B, and Kir6.2/SUR2B. Each data point represents an average of data obtained from 3 to 10 cells.

blockers. For this, we used mitochondrial flavoprotein fluorescence as an indirect index of mito$K_{ATP}$ channel opening in rabbit ventricular myocytes. Diazoxide selectively opens mito$K_{ATP}$ channels but has no effect on cardiac s$K_{ATP}$ channels, whereas pinacidil nonselectively opens both mito$K_{ATP}$ and s$K_{ATP}$ channels (Liu et al., 1998). P-1075 opens cardiac s$K_{ATP}$ but has no effect on mito$K_{ATP}$ channels (Sato et al., 2000). On the other hand, glybenclamide (Jaburek et al., 1998) blocks both mito$K_{ATP}$ and s$K_{ATP}$ channels, whereas 5HD selectively blocks mito$K_{ATP}$ channels in ventricular myocytes (Sato et al., 1998). Recent evidence from our laboratory also shows that HMR-1098 is a selective s$K_{ATP}$ channel blocker which does not block mito$K_{ATP}$ channels (Sato et al., 2000). From Table 1, it is clear that only the coexpression of Kir6.1/SUR1 constructs has a pharmacological profile similar to that of mito$K_{ATP}$ channels in rabbit ventricular myocytes.

We further compared the pharmacological potencies of diazoxide, P-1075, 5HD, and HMR-1098 against heterologously expressed $K_{ATP}$ channels and mito$K_{ATP}$ channels in rabbit ventricular myocytes. Diazoxide activates Kir6.1/SUR1 with an $EC_{50}$ value of 10 $\mu$M (Fig. 3, ■) and mito$K_{ATP}$ channels with an $EC_{50}$ value of 27 $\mu$M (Fig. 3, ○). The latter value is based on measurements of mitochondrial oxidation in intact cells; diazoxide is more potent in activating potassium flux in isolated mitochondria, as shown in the inset of Fig. 3 [replotted from Garlid et al. (1996)]. Thus, the potency with which diazoxide activates Kir6.1/SUR1 membrane current falls well within the range reported for activation of mito$K_{ATP}$ channels. P-1075 opens all the heterologously expressed $K_{ATP}$ channels as shown in Fig. 4, except $K_{ATP}$ channels formed by Kir6.1/SUR1. Interestingly, P-1075 also has no effect on the native mito$K_{ATP}$ channel, as shown in Fig. 4 and by Sato et al. (Sato et al., 2000).

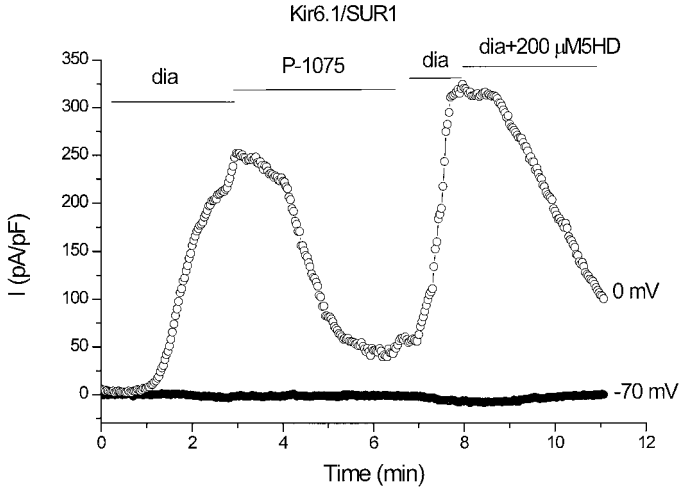


**Fig. 2.** A representative electrophysiological recordings of currents from the Kir6.1/SUR1 channel. ○, $K_{ATP}$ current measured at 0 mV; ●, current measured at −70 mV, which is close to the potassium reversal potential under our experimental conditions and should not change significantly if only a potassium channel is activated. With these constructs, dia (diazoxide; 100 $\mu$M) reversibly activated $K_{ATP}$ channels, whereas P-1075 (100 $\mu$M) did not. 5HD (200 $\mu$M) blocked diazoxide-induced currents.

As shown in Fig. 5, 5HD blocks diazoxide-induced Kir6.1/SUR1 channels with an $IC_{50}$ value of 66 $\mu$M. This value is very close to that required to block native mito$K_{ATP}$ channels ($IC_{50}$ value of 95 $\mu$M). 5HD also blocks Kir6.2/SUR1 ($IC_{50}$ value of 81 $\mu$M). Sato et al. have previously shown that HMR-1098 at 30 $\mu$M completely blocks cardiac s$K_{ATP}$ channels (Sato et al., 2000). We found that HMR1098 potently blocks Kir6.2/SUR2A ($IC_{50}$ of 1.5 $\mu$M) but has no appreciable effect to 30 $\mu$M on Kir6.1/SUR1 or Kir6.2/SUR2B ($IC_{50}$ = 100 $\mu$M) (Fig. 6). HMR-1098 also blocks Kir6.2/SUR1 with an $IC_{50}$ of 5 $\mu$M. HMR-1098 at 30 $\mu$M has no effect on native mito$K_{ATP}$ channels, and has only 20% inhibition at 100 $\mu$M.

## Discussion

Recent evidence has strongly implicated mito$K_{ATP}$ channels as the effectors of IPC and pharmacological cardioprotection (Liu et al., 1999; Szewczyk and Marban, 1999). Mito$K_{ATP}$ channel opening has also been shown to reduce neuronal injury (Domoki et al., 1999). $K_{ATP}$ channels are formed as an octomeric complex of four pore-forming Kir6.x and four sulfonylurea receptors (Aguilar-Bryan et al., 1998). Two subfamilies of Kir (Kir6.1 and Kir6.2) and three subfamilies of SUR (SUR1, SUR2A, and SUR2B) have been identified. Although the exact molecular structure of mito$K_{ATP}$ has not been identified, much is known about its pharmacology. By patch-clamping mitoplasts from rat liver mitochondria, Inoue and coworkers were the first to demonstrate the existence of a mitochondrial potassium channel that is reversibly inactivated by ATP and inhibited by glybenclamide (Inoue et al., 1991). Later, a fraction containing mito$K_{ATP}$ channel activity was purified from the inner membranes of rat liver and beef heart mitochondria (Paucek et al., 1992). Using reconstituted mitochondrial vesicles or isolated mitochondria and measuring potassium flux, Garlid et al. demonstrated that heart and liver mito$K_{ATP}$ channels share some pharmacological properties with the channels found in sarcolemma. However, mitochondrial channels have higher sensitivity to opening by diazoxide, exceeding the sensitivity of sarcolemmal channels by 2000-fold (Garlid et al., 1996). We later confirmed this selectivity to diazoxide in intact rabbit ven-

TABLE 1
Pharmacology of heterologously expressed $K_{ATP}$ channels

| Tissue | Molecular Composition | Dia | Pina | P-1075 | Gly | 5HD | HMR-1098 |
|---|---|---|---|---|---|---|---|
| | Kir6.1/SUR1 | O | O | NE | C | C | NE |
| | Kir6.1/SUR2A | NE | O | O | C | NE | NE |
| VSM | Kir6.1/SUR2B | O | O | O | C | NE | NE |
| Pancreatic | Kir6.2/SUR1 | O | O | O | C | C | C |
| Sarcolemmal | Kir6.2/SUR2A | NE | O | O | C | NE | C |
| VSM | Kir6.2/SUR2B | NE | O | O | C | NE | NE |
| Mitochondria | Unknown | O | O | NE | C | C | NE |

VSM, vascular smooth muscle; Dia, diazoxide (100 $\mu$M); Pina, pinacidil (100 $\mu$M); P-1075 (100 $\mu$M); Gly, glybenclamide (10 $\mu$M); 5HD, 5-hydroxydecanoic acid sodium (200 $\mu$M); HMR-1098 (10 $\mu$M); O, open; C, close; NE, no effect.
The information about tissue specific distribution of $K_{ATP}$ channels was taken from Yokoshiki et al. (1998) and Hu et al. (1999).

tricular myocytes using mitochondrial oxidation as an index of mitoK$_{ATP}$ channel opening (Liu et al., 1998). We further found that pinacidil is a nonselective K$_{ATP}$ channel opener, and P-1075 is a selective cardiac sK$_{ATP}$ channel opener (it does not open mitoK$_{ATP}$ channels) in ventricular myocytes (Liu et al., 1998; Sato et al., 2000). We also have shown that 5HD and HMR-1098 selectively block mitoK$_{ATP}$ and sK$_{ATP}$ channels, respectively, in ventricular myocytes (Sato et al., 1998, 2000). The mitoK$_{ATP}$ channel has not been cloned, although several observations suggest that mitoK$_{ATP}$ channels contain both the Kir6.x subunit (Suzuki et al., 1997) and the SUR subunit (because of its sensitivity to glybenclamide). We thus studied the pharmacology of all six known types of K$_{ATP}$ channels heterologously expressed in HEK293 cells and compared it with that of mitoK$_{ATP}$ channels.

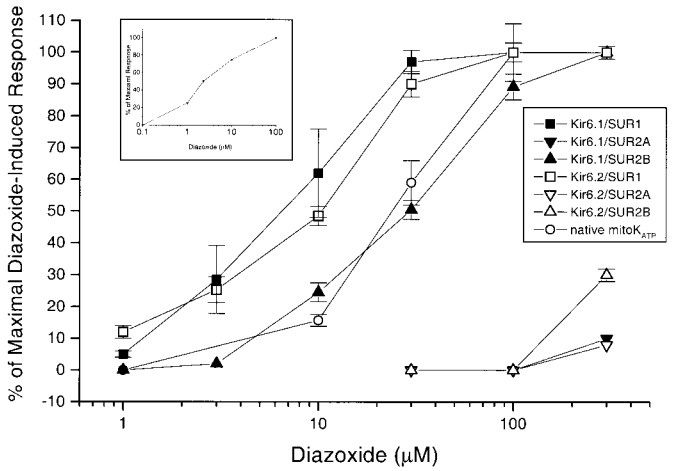


**Fig. 3.** A comparison of the potency of diazoxide to activate K$_{ATP}$ channels heterologously expressed in HEK293 cells and native mitoK$_{ATP}$ channels in rabbit ventricular myocytes. For heterologously expressed channel Kir6.1/SUR1, Kir6.1/SUR2B, and Kir6.2/SUR1, the dose-response curves were constructed as a percentage of 300 $\mu$M diazoxide-induced K$_{ATP}$ currents. For heterologously expressed channel Kir6.1/SUR2A, Kir6.2/SUR2A, and Kir6.2/SUR2B, the dose-response curves were constructed as a percentage of 100 $\mu$M pinacidil-induced K$_{ATP}$ currents. To obtain the dose-response curve in the native mitoK$_{ATP}$ channel in rabbit ventricular myocytes, we measured mitochondrial flavoprotein fluorescence and plotted the response as a percentage to the maximal effect of 300 $\mu$M diazoxide. For the clarity of comparison, we also re-plot the data of potency to induce potassium flux in isolated mitochondria from a study by Garlid et al. (1996) in the inset. Each data point is the average of the data from 2 to 10 cells.

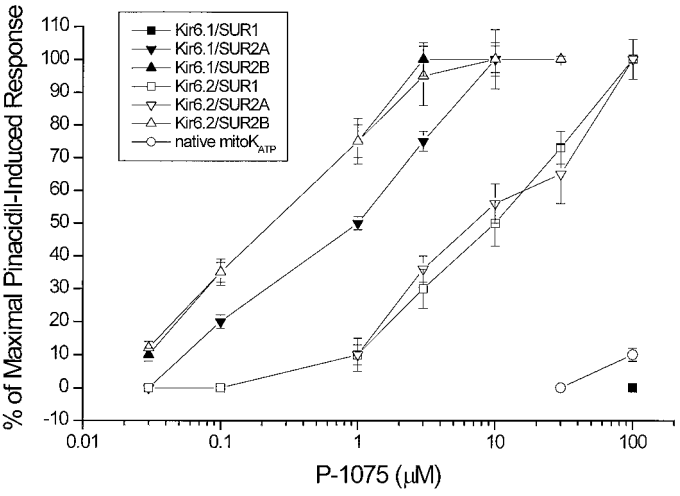


**Fig. 4.** A comparison of the potency of P-1075 to activate K$_{ATP}$ channels heterologously expressed in HEK293 cells and native mitoK$_{ATP}$ channels in rabbit ventricular myocytes. The dose-response curves for heterologously expressed K$_{ATP}$ channels were obtained by plotting a percentage of P-1075-induced currents to 100 $\mu$M pinacidil-induced K$_{ATP}$ currents. For native mitoK$_{ATP}$ channels in rabbit ventricular myocytes, the dose-response curve was then constructed as a percentage of flavoprotein fluorescence increase induced by P-1075 to those from 100 $\mu$M pinacidil. Each data point is the average of the data from 2 to 10 cells.

**Comparison of Sensitivity to K$_{ATP}$ Channel Openers.** Pinacidil at 100 $\mu$M opens all six types of K$_{ATP}$ channels (Kir6.1/SUR1, Kir6.2/SUR1, Kir6.1/SUR2A, Kir6.2/SUR2B, Kir6.1/SUR2B, and Kir6.2/SUR2B). Consistent with our previous study on intact rabbit ventricular myocytes (Liu et al., 1998), diazoxide did not open Kir6.2/SUR2A cardiac-type sK$_{ATP}$ channels. Diazoxide at 100 $\mu$M also failed to activate

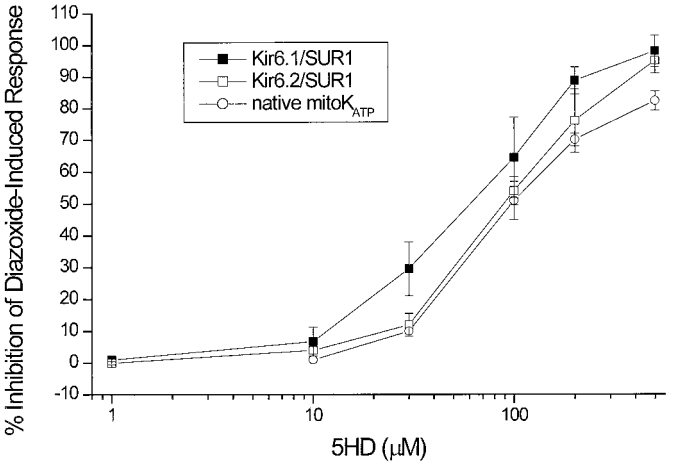


**Fig. 5.** A comparison of the potency of 5HD to inhibit heterologously expressed Kir6.1/SUR1 and Kir6.2/SUR1 channels (the only two 5HD-inhibitable K$_{ATP}$ channels), and native mitoK$_{ATP}$ channels in rabbit ventricular myocytes. The 5HD dose-response curves for Kir.6.1/SUR1 and Kir6.2/SUR1 channels were obtained by plotting the percentage reduction to 100 $\mu$M diazoxide-induced K$_{ATP}$ currents by 5HD. For native mitoK$_{ATP}$ channels in rabbit ventricular myocytes, the dose-response curve was then constructed with the percentage inhibition by 5HD of 100 $\mu$M diazoxide-induced flavoprotein fluorescence oxidation. Each data point is the average of the data from 2 to 10 cells.

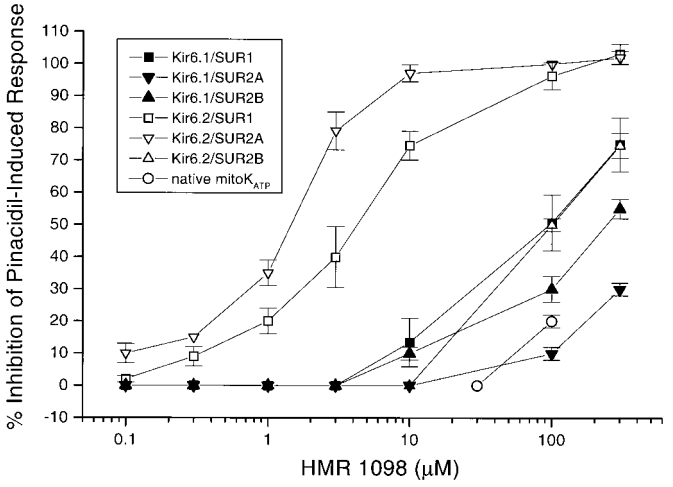


**Fig. 6.** A comparison of the potency of HMR-1098 to inhibit all six types of heterologously expressed K$_{ATP}$ channels in HEK293 cells. The dose-response curves were plotted as the percentage reduction of 100 $\mu$M pinacidil-activated K$_{ATP}$ currents by HMR-1098. Pinacidil was used in this study, because it activated all six types of K$_{ATP}$ channels. Each data point is average of the data from 2–10 cells.

Kir6.2/SUR2B, one of the proposed vascular $K_{ATP}$ channels (Isomoto et al., 1996). But, at a higher concentration (200 $\mu$M), diazoxide does open this channel (Fig. 3), consistent with a previous study in cell-attached patches (Isomoto et al., 1996). Diazoxide has been shown to be slightly more potent in opening this channel in excised patches (Schwanstecher et al., 1998) (at 100 $\mu$M, diazoxide elicited 75% of maximal channel activity), but this difference may have been caused by the excised patch versus intact cell in our study. Diazoxide does activate Kir6.1/SUR2B, another vascular $K_{ATP}$ channel that responds to $K_{ATP}$ channel openers and glybenclamide but is insensitive to ATP (Yamada et al., 1997). As shown in Fig. 4, P-1075 is a potent opener for the smooth muscle $K_{ATP}$ channels (Kir6.1/SUR2B and Kir6.2/SUR2B), with an $EC_{50}$ value of 0.16 $\mu$M. P-1075 also activates Kir6.1/SUR2A, Kir6.2/SUR1 (pancreatic $\beta$-cell), and Kir6.2/SUR2A. Interestingly, P-1075 had no effect on either Kir6.1/SUR1 or native mito$K_{ATP}$ channels.

**Comparison of Sensitivity to $K_{ATP}$ Channel Blockers.** Glybenclamide is a potent and nonselective $K_{ATP}$ channel blocker; 10 $\mu$M blocked all six types of the reconstituted $K_{ATP}$ channels. Although we could not evaluate the effects of glybenclamide on native mito$K_{ATP}$ channels in rabbit ventricular myocytes using flavoprotein fluorescence as an indirect index [because of an apparent uncoupling effect (Szewczyk et al., 1997)] (Sato et al., 1998), Jaburek et al. (1998) demonstrated, using potassium flux measurement in isolated mitochondria, that glybenclamide blocks mito$K_{ATP}$ channels with a $K_{1/2}$ value of 1 to 6 $\mu$M (Jaburek et al., 1998). Interestingly, 5HD (at 200 $\mu$M) blocks only the $K_{ATP}$ channels formed by Kir6.1/SUR1 and Kir6.2/SUR1. It does not block cardiac-type s$K_{ATP}$ (Kir6.2/SUR2A), consistent with results in cardiac ventricular myocytes (Sato et al., 1998). Our results with HMR-1098 on heterologously expressed $K_{ATP}$ channels are also consistent with its reported pharmacology in native cells. HMR-1098 blocked Kir6.2/SUR2A in this study, whereas it blocks s$K_{ATP}$ in rabbit ventricular myocytes (Sato et al., 2000). HMR-1883, a lipophilic derivative of HMR-1098, also completely blocked s$K_{ATP}$ channels in guinea pig ventricular myocytes (Gogelein et al., 1998). HMR-1098 did not block Kir6.2/SUR2B (a vascular $K_{ATP}$ channel) in this study: consistent with this finding, much higher concentrations of HM-1883 are required to inhibit coronary vasodilation induced by hypoxia in the guinea pig (Gogelein et al., 1998). HMR-1098 up to 10 $\mu$M also had no inhibitory effect on Kir6.1/SUR2B, another $K_{ATP}$ channel found in vascular smooth muscle cells (Yamada et al., 1997). An intermediate concentration of HMR-1098 is required to inhibit Kir6.2/SUR1, a pancreatic $\beta$-cell type $K_{ATP}$ channel, also consistent with the study of HMR-1883 in native pancreatic cells (Gogelein et al., 1998).

**Pharmacological Similarities of $K_{ATP}$ Channels Formed by Kir6.1/SUR1 to mito$K_{ATP}$ Channels.** Based on the data summarized in Table 1, it is clear that channels formed by Kir6.1/SUR1 coexpression pharmacologically resemble mito$K_{ATP}$ channels. This similarity is further illustrated by the following comparisons.

Diazoxide activates Kir6.1/SUR1 channels with an $EC_{50}$ value of 10 $\mu$M and native mito$K_{ATP}$ channels in rabbit ventricular myocytes with an $EC_{50}$ value of 27 $\mu$M (Fig. 3). The $EC_{50}$ value of 10 $\mu$M to activate Kir6.1/SUR1 is close to the value that is reported on isolated mitochondria [$K_{1/2}$, 2.3 $\mu$M (Garlid et al., 1996)]. This value is also not inconsistent with the 27 $\mu$M to half-maximal activation of mito$K_{ATP}$ channels in intact myocytes (Fig. 3), considering the diffusion barriers between extracellularly applied diazoxide and the mitochondria in intact myocytes and other differences in the experimental conditions. Although P-1075 activates most of the heterologously expressed $K_{ATP}$ channels, it has no effect on Kir6.1/SUR1 or native mito$K_{ATP}$ channels up to 100 $\mu$M. Furthermore, channels formed by Kir6.1/SUR1 have very similar profiles to pharmacological blockade as well. 5HD blocks Kir6.1/SUR1 channels and mito$K_{ATP}$ channels with similar potency (Fig. 4). 5HD has been reported to block mito$K_{ATP}$ channels reconstituted in liposomes and in isolated mitochondria with a $K_{1/2}$ value of 58 to 85 $\mu$M (Jaburek et al., 1998). Sato et al. have reported that HMR-1098 at 30 $\mu$M completely inhibits cardiac s$K_{ATP}$ channels, but does not block the native mito$K_{ATP}$ channel in rabbit ventricular myocytes (Sato et al., 2000). At 10 $\mu$M, HMR-1098 does not significantly inhibit Kir6.1/SUR1 channels, and it causes only about 50% inhibition at 100 $\mu$M. However, HMR-1098 potently inhibits Kir6.2/SUR2A, and the potency ($IC_{50}$ = 1.5 $\mu$M) is very similar to that of HMR-1883 (a lipophilic derivative of HMR-1098) on inhibition of s$K_{ATP}$ channels in guinea pig ventricular myocytes ($IC_{50}$ = 0.8 $\mu$M) (Gogelein et al., 1998).

Taken together, we have demonstrated that the $K_{ATP}$ channel formed by the coexpression of Kir6.1 and SUR1 has a pharmacological profile similar to that of native mito$K_{ATP}$ channels. However, this does not necessarily imply that Kir6.1/SUR1 represents the mito$K_{ATP}$ channel. Kir6.1 gene has a ubiquitous expression profile with higher levels in the heart (Inagaki et al., 1995; Akao et al., 1997). In addition, immunogold staining has localized Kir6.1 to the inner mitochondrial membrane in skeletal muscle (Suzuki et al., 1997). Nevertheless, using a viral gene transfer technique, we did not observe any significant suppression of diazoxide-induced mito$K_{ATP}$ channel opening by a dominant-negative Kir6.1 construct in rabbit ventricular myocytes, and Kir6.1 antibody staining was not colocalized with mitochondria in isolated cardiac myocytes (Seharaseyon et al., 2000). Furthermore, the sizes of the mitochondrial Kir and SUR purified from isolated mitochondria are not consistent with the conventional $K_{ATP}$ channel subunits (Paucek et al., 1999). Despite these negative findings, mito$K_{ATP}$ channels may contain structural motifs involved in drug binding similar to that of Kir6.1/SUR1, perhaps providing a clue to the ultimate resolution of the structure of this channel. Although more studies are needed to identify the molecular structure of the mito$K_{ATP}$ channels, the present findings are the first step in defining the molecular correlates of drug sensitivity for mito$K_{ATP}$ channels and can be used as a guide for future structure-function analyses.

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