ATP-Sensitive Potassium (K_{ATP}) Channel Activation Decreases Intraocular Pressure in the Anterior Chamber of the Eye

Uttio Roy Chowdhury,¹ Cindy K. Bahler,¹ Cheryl R. Hann,¹ Minhwang Chang,² Zachary T. Resch,¹ Michael F. Romero,² and Michael P. Fautsch¹

PURPOSE. ATP-sensitive potassium channel (K_{ATP}) openers target key cellular events, many of which have been implicated in glaucoma. The authors sought to determine whether K_{ATP} channel openers influence outflow facility in human anterior segment culture and intraocular pressure (IOP) in vivo.

METHODS. Anterior segments from human eyes were placed in perfusion organ culture and treated with the K_{ATP} channel openers diazoxide, nicorandil, and P1075 or the K_{ATP} channel closer glyburide (glibenclamide). The presence, functionality, and specificity of K_{ATP} channels were determined by RT-PCR, immunohistochemistry, and inside-out patch clamp in human trabecular meshwork (TM) tissue or primary cultures of normal human trabecular meshwork (NTM) cells. The effect of diazoxide on IOP in anesthetized Brown Norway rats was measured with a rebound tonometer.

RESULTS. K_{ATP} channel openers increased outflow facility in human anterior segments (0.14 ± 0.02 to 0.26 ± 0.09 $\mu\text{L}/\text{min}/\text{mm Hg}$; $P < 0.001$) compared with fellow control eyes (0.22 ± 0.11 to 0.21 ± 0.11 $\mu\text{L}/\text{min}/\text{mm Hg}$; $P > 0.5$). The effect was reversible, with outflow facility returning to baseline after drug removal. The addition of glyburide inhibited diazoxide from increasing outflow facility. Electrophysiology confirmed the presence and specificity of functional K_{ATP} channels. K_{ATP} channel subunits $K_{ir}6.1$, $K_{ir}6.2$, SUR2A, and SUR2B were expressed in TM and NTM cells. In vivo, diazoxide significantly lowered IOP in Brown Norway rats.

CONCLUSIONS. Functional K_{ATP} channels are present in the trabecular meshwork. When activated by K_{ATP} channel openers, these channels increase outflow facility through the trabecular outflow pathway in human anterior segment organ culture and decrease IOP in Brown Norway rat eyes. (*Invest Ophthalmol Vis Sci.* 2011;52:6435–6442) DOI:10.1167/iows.11-7523

The juxtacanalicular region of the trabecular meshwork (TM) and the basement membrane of Schlemm's canal inner wall endothelium are considered the primary sites re-

sponsible for increased outflow resistance to aqueous humor drainage from the human anterior chamber. The increase in resistance (decrease in outflow facility) in this area leads to elevated intraocular pressure (IOP), the most prevalent risk factor for primary open-angle glaucoma (POAG). An increase in trabecular meshwork (TM) cell contractility, changes in permeability, cell volume, and different forms of stress are a few of the mechanisms believed to increase outflow resistance in the trabecular outflow pathway.^{1–11} However, it is unknown what molecular components of TM cells are involved in modulating these mechanisms.

Potassium channels are the most diverse of all the ion transporters. Adenosine triphosphate-sensitive potassium (K_{ATP}) channels are unique inward-rectifying potassium channels that connect the metabolic state of the cell to membrane excitability because of their characteristic inhibition by micromolar concentrations of intracellular ATP.¹² K_{ATP} channels are prominently involved in the regulation of insulin secretion from the pancreatic beta cells, glucose homeostasis in the hypothalamus, cardioprotection, and cellular adaptation to stress.^{10,13–16} The structure of K_{ATP} channels is complex, cell-type specific, and, in the case of mitochondria and endoplasmic reticulum, organelle specific. Although most potassium (K^+) channels have a tetrameric structure, K_{ATP} channels are octamers, made up of a tetrameric channel pore consisting of a potassium inwardly rectifying subunit ($K_{ir}6.1$ or $K_{ir}6.2$) surrounded by a tetrameric shell containing sulfonylurea receptor subunits (SUR1, SUR2A, or SUR2B). Different channel subunit combinations are expressed selectively within tissues. For example, $K_{ir}6.1/\text{SUR1}$ channels are found predominantly in pancreatic beta cells, $K_{ir}6.2/\text{SUR2B}$ channels in nonvascular smooth muscle cells, $K_{ir}6.1/\text{SUR2B}$ channels in endothelial and vascular smooth muscle cells, and $K_{ir}6.2/\text{SUR2A}$ channels in cardiac and skeletal muscle.^{17–21} In mitochondria, several K_{ATP} channel subunit combinations have been identified containing either $K_{ir}6.1$ or $K_{ir}6.2$.^{22–29}

Pharmaceutical agents that open or close K_{ATP} channels have important clinical applications. K_{ATP} channel openers diazoxide and nicorandil are used to treat acute hypertension and angina,^{30,31} whereas K_{ATP} channel closer glyburide (glibenclamide) is used to treat type 2 diabetes.³² In nonocular cells, the opening and closing of K_{ATP} channels has been shown to modulate cellular contractility, cell adhesion, and permeability and provides metabolic protection against ischemia and hypoxia.^{4–6,33–37} In addition, K_{ATP} channels affect gap and tight junction regulation, enhance cellular adaptation to stress (shear, stretch, pressure, and oxidation), and improve overall cell well-being.^{11,16,38–48} Interestingly, all these processes have been implicated in altering outflow facility in the eye and therefore in the pathophysiology of POAG. However, the role of K_{ATP} channels in ocular tissues has never been studied. We reasoned that in light of this functional relevance

From the Departments of ¹Ophthalmology and ²Physiology and Biomedical Engineering, Mayo Clinic, Rochester, Minnesota.

Supported in part by National Institutes of Health Research Grants EY 15736 (MPF), EY 07065 (MPF), and EY 017732 (MFR); the Mayo Foundation; and Research to Prevent Blindness (Lew R. Wasserman Merit Award [MPF] and an unrestricted grant [Department of Ophthalmology, Mayo Clinic]).

Submitted for publication March 8, 2011; revised May 13 and June 8, 2011; accepted June 23, 2011.

Disclosure: U.R. Chowdhury, None; C.K. Bahler, None; C.R. Hann, None; M. Chang, None; Z.T. Resch, None; M.F. Romero, None; M.P. Fautsch, None

Corresponding author: Michael P. Fautsch, Department of Ophthalmology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905; fautsch.michael@mayo.edu.

of K_{ATP} channels to the underlying events leading to POAG, these channels may also have a role in IOP regulation. In the present study we evaluated the effect of various pharmacologic K_{ATP} channel openers on outflow facility in an ex vivo human anterior segment eye culture model, the specificity of the response by patch clamp, and the in vivo effect on IOP in Brown Norway rats.

METHODS

Anterior Segment Culture

The use of donor human eyes for this study was approved by the Mayo Clinic Institutional Review Board and conformed to the tenets of the Declaration of Helsinki. Fourteen pairs of normal human eyes from six male and eight female donors (75 ± 10 years, mean $\pm$ SD; age range, 58–96 years) were obtained from the Minnesota Lions Eye Bank and were placed in anterior segment perfusion culture within 12.4 ± 5.5 hours of death. No eyes had glaucoma or were from patients receiving topical eye medications. The culture technique was similar to that described previously.^{49–52} Eyes were bisected at the equator, and the iris, lens, and vitreous were removed. The anterior segment was clamped in a modified Petri dish, and the eye was perfused with Dulbecco's modified Eagle's medium (DMEM; Mediatech, Inc., Manassas, VA) containing antibiotics (penicillin, 10,000 U; streptomycin, 10 mg; amphotericin B, 25 mg; and gentamicin, 1.7 mg in 100 mL medium). Anterior segments were maintained at 37°C in a 5% CO₂ atmosphere while being perfused at the normal human flow rate (2.5 μ L/min). Pressure (mm Hg) was continuously monitored in real time with a pressure transducer connected to a second access cannula built into the modified Petri dish and was recorded with an automated computerized system.

Study 1: Anterior Segments Perfused with K_{ATP} Channel Openers. In eight pairs of eyes, one anterior segment received DMEM containing 20 μ M diazoxide (dissolved in dimethyl sulfoxide [DMSO]) while the fellow anterior segment received vehicle control (DMSO) by anterior chamber (AC) exchange. The concentration of diazoxide (20 μ M) was chosen based on dose-response studies with 5 μ M, 10 μ M, and 20 μ M. A search of the diazoxide literature confirmed concentrations used for in vitro or in vivo studies ranged from 1 to 100 μ M.^{53–55} The AC exchanges were performed using a gravity-driven, constant pressure method over a 5-minute period. After AC exchange, anterior segments were continuously perfused with diazoxide or vehicle for up to 10 days. In 6 of the 8 pairs of anterior segments, drug and vehicle were removed and replaced with DMEM alone to see whether pressure returned to baseline.

In four additional anterior segments, K_{ATP} channel openers nicorandil (20 μ M, $n = 2$) and P1075 (100 μ M, $n = 2$) were added by AC exchange. The fellow anterior segments all received vehicle (DMSO). After the initial AC exchange, anterior segments were continuously perfused with drug or vehicle for up to 3 days.

Outflow facility (C) was calculated at 0 hours (C_0) and 24 hours (C_d) after K_{ATP} channel opener treatment by dividing the flow rate (2.5 μ L/min) by the pressure reading (mm Hg). Outflow facility was plotted as percentage of C and obtained by $[(C_d/C_0) - 1] \times 100$. The outflow facility of the treated anterior segments were grouped and compared with the control anterior segments outflow facility. Group means and

standard deviations were calculated. Significance analysis was performed by a paired, two-tailed Student's *t*-test. Differences were considered significant when $P < 0.05$.

Study 2: Anterior Segments Perfused with Diazoxide and K_{ATP} Channel Closer Glyburide. In three pairs of eyes, one anterior segment received DMEM containing diazoxide (20 μ M, dissolved in DMSO) and glyburide (5 μ M, dissolved in DMSO), whereas the fellow anterior segment received only diazoxide (20 μ M) by AC exchange. After initial AC exchange and continuous perfusion for 24 hours, drugs were removed and replaced with DMEM. A minimum of 24 hours later, a second AC exchange followed by constant perfusion was performed in a crossover manner. The anterior segment that originally received diazoxide (20 μ M) and glyburide (5 μ M) now received diazoxide (20 μ M) alone. The other anterior segment that initially received diazoxide alone now received diazoxide (20 μ M) and glyburide (5 μ M). After crossover, cultures were maintained for up to 48 hours.

Outflow facility and percentage change in outflow facility was calculated as described in Study 1. The outflow facilities of the diazoxide-treated anterior segments were grouped and compared with the outflow facilities of diazoxide- and glyburide-treated anterior segments. Significance of grouped means was determined by a paired, two-tailed Student's *t*-test.

Histologic Examination

At the completion of the anterior segment culture experiments, selected wedges of tissue 180° apart that included the TM and Schlemm's canal were isolated and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2). Light microscopy examination was performed on all cultured anterior segments to assess the TM cells for toxicity. Tissue wedges were embedded in JB4 plastic, sectioned at 3 μ m, and stained with toluidine blue. Examination was performed in a masked fashion using predetermined criteria as described previously.⁵² TM was considered normal if numerous trabecular cells were observed, cells remained in their normal position on the lamellae, and no minor or major disruptions of the juxtacanalicular tissue and trabecular lamellae were seen. Transmission electron microscopy was performed on sections isolated from both anterior segments in 8 of the 14 pairs of eyes.

Expression of K_{ATP} Channel Subunits in Human TM and Normal Primary Human TM Cells

RNA was isolated from dissected human TM from normal donor eyes and confluent human primary normal TM (NTM) cells using a kit (RNeasy Mini; Qiagen, Valencia, CA) in accordance with the manufacturer's protocol. Total RNA (250 ng) was reverse transcribed into cDNA using a cDNA synthesis kit (iScript; Bio-Rad, Hercules, CA). After reverse transcription, a PCR reaction mix of cDNA was prepared using DNA polymerase and master mix (HotStar Taq; Qiagen) in accordance with the manufacturer's protocol, with specific primers against the various K_{ATP} subunits (Table 1). Subsequently, cDNA was amplified in a thermal cycler (Perkin Elmer, Waltham, MA) with the following cycles: initial activation at 95°C for 15 minutes; 35 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, extension at 72°C for 1 minute; and final extension of the PCR products at 72°C for 10 minutes. The PCR products were separated on a 1.5% agarose gel and photographed

TABLE 1. Sequences of Primers Used for Amplification of K_{ATP} Channel Subunits

Gene	5' Primer	3' Primer	Product Length (base pairs)
<i>SUR1</i>	ACTGGATGGTGAGGAACCTGGC	TGGATCTGGATCTTCCTTG	157
<i>SUR2A</i>	GTCACCTGAAGGTGGGGAGAA	GGCTGCAAGGCTGTCATTACT	169
<i>SUR2B</i>	CTGCCACCACTAACTCGTCATC	GTAGGGACATGGATGAAACTG	226
<i>K_{ir}6.1</i>	AGTTGGTGAACCCAGATCGC	CAGTTTTCGCTCAGGCTTC	159
<i>K_{ir}6.2</i>	GCTTGGGGGTGGATATCTTTG	CAACGGAGAAGGCAGAGTTTC	173

(Gel Doc System; Bio-Rad, Hercules, CA). PCR products were isolated using Qiagen PCR purification kit, and DNA sequencing was performed to validate K_{ATP} channel subunits.

Immunohistochemistry of K_{ATP} Subunits in Human TM and Primary Human NTM Cells

For immunohistochemistry (IHC) of human TM, tissue wedges containing the TM region from fresh donor eyes were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2), dehydrated in a graded series of ethanol (75%, 85%, 95%, and 100%) and embedded in paraffin. Sections (5 μ m) were mounted on glass slides (Superfrost/Plus; Fisher, Pittsburgh, PA) and baked at 60°C for 2 hours. For IHC, tissue sections were deparaffinized in xylene and rehydrated in a graded series of ethanol (100%, 95%, 80%, and 70%). Antigens were retrieved by incubating sections at 95°C in 1 mM EDTA (pH 8.0).⁴⁹ Tissue sections were blocked in phosphate-buffered saline (PBS) containing 3% bovine serum albumin (BSA) and 0.1% Triton X-100 and were probed with the following antibodies: human SUR1 mouse monoclonal (Novus Biologicals, Littleton CO), SUR2A goat polyclonal (Santa Cruz Biotechnology, Santa Cruz, CA), SUR2B goat polyclonal (Santa Cruz Biotechnology), K_{ir}6.1 rabbit polyclonal (Abcam, Cambridge, MA), and K_{ir}6.2 rabbit polyclonal (Novus Biologicals). Alexa Fluor 488 conjugated anti-mouse, Alexa Fluor 488 conjugated anti-goat, and Alexa Fluor 546 conjugated anti-rabbit IgGs (Molecular Probes, Eugene, OR) were used as secondary antibodies. Sections were mounted in mounting medium (Vectashield/DAPI; Vector Laboratories, Burlingame, CA) and were analyzed with a confocal laser microscope (Zeiss 510; Carl Zeiss, Thornwood, NY). Sections incubated with secondary antibody alone were used as negative controls.

For human primary NTM monolayers, cells were grown to confluence on eight-well chamber slides, fixed in 4% paraformaldehyde for 10 minutes, and blocked in 1% bovine serum albumin in PBS. Cells were incubated with the same panel of primary and secondary antibodies as outlined for TM. After secondary antibody incubation and subsequent washes, the chamber partitions were removed, and the slides were mounted in mounting medium (Vectashield/DAPI; Vector Laboratories). Slides were examined and photographed using a confocal laser microscope (Zeiss 510; Carl Zeiss). As with the TM tissue, cells incubated with secondary antibody alone were used as negative controls.

Patch Clamp

Single-channel activity was recorded in the inside-out patch configuration on human primary NTM cells seeded onto coverslips. Standard patch clamp experiments were carried out on the stage of an inverted microscope (IX71; Olympus, Center Valley, PA) at room temperature (20°C–23°C). All experiments occurred within 3 days after seeding the suspension of NTM cells onto the coverslips. The membrane patches were directly exposed to control or test solutions by a multivalve perfusion system (Warner Instruments, Hamden, CT) in a low profile perfusion chamber (Bioscience Tool, San Diego, CA). Patch pipettes were pulled using borosilicate glass (World Precision Instruments, Inc., Sarasota, FL), and pipettes were back filled with a pipette control solution (140 mM KCl, 10 mM HEPES, 1.4 mM MgCl₂, 1 mM EGTA, 10 mM glucose^{56,57} and pH adjusted to 7.35 with KOH). This solution with 5 mM EGTA was also used in the recording chamber to superfuse the cells/patches for symmetrical [K⁺] experiments. Pipette resistances ranged from 2 to 6 mega-ohms when filled with pipette solution. Once a giga-ohm seal was formed, C-Fast compensation circuit was used to cancel fast capacitive currents, and inside-out patches were excised from the membrane of NTM cells.

Voltage-clamp recording was carried out using a patch-clamp amplifier (EPC10-Plus; HEKA Elektronik, Lambrecht/Pfalz, Germany) controlled by acquisition software (PatchMaster; HEKA Elektronik). Recordings were obtained from patches treated with diazoxide (100 μ M), diazoxide (100 μ M) and glyburide (200 μ M) or buffer alone.

Single-channel activities were recorded in response to 500-ms voltage steps (20 mV) from –140 to +60 mV (holding potential, –60 mV). Currents were filtered at 1 kHz with a four-pole, low-pass Bessel filter, sampled at 2 to 20 kHz and stored directly into the computer's hard drive. All records were inspected visually, followed by automatic channel event detection using analysis software (FitMaster; HEKA Elektronik). Amplitude histograms were fitted with Gaussian distribution using Simplex fit algorithm. The channel opening probability (P_o) was determined from the ratio of the area under the peak estimated from the Gaussian fitted histograms.⁵⁶ The mean single channel current magnitude was estimated using power spectrum analysis. Data from five different patches are expressed as mean $\pm$ SD.

Measurement of IOP in Brown Norway Rats

The use of Brown Norway rats for this study was approved by the Mayo Institutional Animal Care and Use Committee and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Adult male Brown Norway rats (retired breeders, 325–375 g, $n = 12$) were purchased from Charles River Laboratories International, Inc (Wilmington, MA). The rats were anesthetized by intraperitoneal injection of thio butabarbital sodium salt hydrate (Inactin; Sigma-Aldrich, St. Louis, MO) dissolved in PBS (100 mg/kg). Baseline IOP of both right and left eyes of each animal was measured with a rebound tonometer (TonoLab; Colonial Medical Supply, Franconia, NH) at 20, 40, and 60 minutes before treatment. The average of the three readings for each eye was used as the baseline IOP. The tonometer (TonoLab; Colonial Medical Supply) was held horizontally, and the probe contacted the eye perpendicularly to the central cornea, in accordance with the manufacturer's protocol. For treating the eyes, a 100-mM stock solution of diazoxide (in DMSO) was diluted 20-fold in 10% nonionic polyethoxylated detergent (Cremophor EL; Sigma-Aldrich) for a final working concentration of 5 mM. Ten microliters of the 5-mM diazoxide solution was used for one eye of each animal. The same volume of vehicle (DMSO in 10% nonionic polyethoxylated detergent) was added to the fellow eye and served as the control. Treated and control eyes were selected randomly. IOP was measured every 20 minutes over a 4-hour period. For each eye, IOP data from 20, 40, and 60 minutes posttreatment were averaged and presented as 1-hour data. The same was done for the 2-, 3-, and 4-hour data. Final IOP of treated eyes was expressed as percentage change compared with the control eye. Data were compared using Student's paired *t*-test, with differences considered significant when $P < 0.05$.

RESULTS

K_{ATP} Channel Openers and Outflow Facility

To test whether K_{ATP} channel openers have an effect on outflow facility, we treated human anterior segments with diazoxide, nicorandil, and P1075. These K_{ATP} channel openers selectively recognize the SUR subunit of K_{ATP} channels. Treatment of human anterior segments with diazoxide (20 μ M) decreased pressure within 24 hours from 17.6 ± 3.6 mm Hg to 10.4 ± 3.7 mm Hg ($n = 8$; $P < 0.0001$) and increased outflow facility from 0.15 ± 0.03 μ L/min/mm Hg to 0.27 ± 0.11 μ L/min/mm Hg ($n = 8$; $P < 0.008$; Fig. 1A, representative graph). All eight anterior segments treated with diazoxide showed an increase in outflow facility at 24 hours posttreatment (Fig. 1B). In contrast, the fellow anterior segments treated with the vehicle (DMSO) had no change in pressure (14.6 ± 9.6 mm Hg to 15.1 ± 8.8 mm Hg; $P > 0.5$) and outflow facility (0.22 ± 0.11 μ L/min/mm Hg to 0.21 ± 0.12 μ L/min/mm Hg; $P > 0.5$; Fig. 1B). The effect of diazoxide was reversible, with outflow facility returning to baseline after drug removal. Treatment of two pairs of anterior segments with nicorandil also increased outflow facility (0.14 ± 0.01 μ L/min/mm Hg to 0.21 ± 0.03 μ L/min/mm Hg), as did treatment of two pairs of human anterior segments with P1075 ($0.14 \pm$

FIGURE 1. K_{ATP} channel openers increase outflow facility in human anterior segment culture. (A) Representative pressure graph indicating a decrease in IOP after the addition of 20 μ M diazoxide to perfusate. Removal of diazoxide returned IOP to baseline. (B) Change in outflow facility from baseline to 24 hours posttreatment with diazoxide (eye pairs 1–8), nicorandil (eye pairs 9–10), and P1075 (eye pairs 11–12). Control eyes were all treated with vehicle control (DMSO). Bars labeled Mean represent mean outflow facility for all 12 eye pairs.

0.01 μ L/min/mm Hg to 0.23 ± 0.07 μ L/min/mm Hg; Fig. 1B). Considering all combined measurements, treatment with K_{ATP} channel openers ($n = 12$) increased outflow facility from 0.14 ± 0.02 μ L/min/mm Hg to 0.26 ± 0.09 μ L/min/mm Hg ($P < 0.001$), whereas treatment with vehicle did not change outflow facility (0.22 ± 0.11 μ L/min/mm Hg to 0.21 ± 0.11 μ L/min/mm Hg; $P > 0.5$).

On histologic examination, eyes treated with diazoxide, nicorandil, or P1075 appeared normal, displaying viable TM cells and an intact Schlemm's canal inner and outer wall with healthy appearing cells (Fig. 2, 10-day diazoxide treatment). It should be noted that in several eye pairs, the anterior segments treated with vehicle (DMSO) had some swollen and rounded cells, with a few minor breaks in the inner and outer walls of Schlemm's canal indicating a toxic effect of DMSO. Given the observation that all anterior segments treated with K_{ATP} channel openers appeared normal, this indicated that K_{ATP} channel activation may have a protective role for the cells of the outflow pathway.

To further analyze the effect of K_{ATP} channels on outflow facility, the K_{ATP} channel closer glyburide (5 μ M) was added with diazoxide (20 μ M) to human anterior segments. Using a crossover experiment, anterior segments ($n = 6$) treated with diazoxide increased outflow facility from 0.14 ± 0.02 μ L/min/mm Hg to 0.33 ± 0.14 μ L/min/mm Hg ($P < 0.01$). Alternatively, eyes treated simultaneously with both diazoxide and glyburide had no appreciable change in outflow facility (0.16 ± 0.02 μ L/min/mm Hg to 0.16 ± 0.03 μ L/min/mm Hg, $n = 6$; $P > 0.85$; Fig. 3).

FIGURE 2. Histology of TM after treatment with diazoxide. (A) JB4-sectioned, toluidine blue-stained images of a representative anterior segment pair treated with diazoxide or vehicle for 10 days. (B) Transmission electron micrographs of the same eye pair. SC, Schlemm's canal; JCT, juxtacanalicular region.

Activation of K_{ATP} Channels in Human Primary NTM Cells

To determine whether functional K_{ATP} channels exist in human primary NTM cells, inside-out patch clamp was performed (Fig. 4). The addition of control buffer to NTM membrane patches by a multivalve perfusion system showed minimal single-channel inward-rectifying activity over the 20-mV step gradient from -140 to $+60$ mV. The addition of diazoxide (100 μ M) to the control buffer increased K_{ATP} channel activity by opening numerous channels for greater lengths of time. The channel opening probability (P_o) at -60 mV increased from 0.29 ± 0.05 in the control buffer to 0.62 ± 0.05 with the addition of diazoxide. In addition, the current magnitude increased from 1.68 ± 0.29 pA to 3.34 ± 0.35 pA after diazoxide treatment. K_{ATP} channel activity was attenuated after the addition of glyburide ($P_o = 0.31 \pm 0.03$ at -60 mV). These patch clamp studies indicate that K_{ATP} channels are present in human primary NTM cells and are capable of responding to both K_{ATP} channel openers and K_{ATP} channel closers.

K_{ATP} Channel Subunits within the TM

Subunit composition of K_{ATP} channels influences functional aspects of the channel. To determine which K_{ATP} channel

FIGURE 3. Glyburide inhibits diazoxide effect on outflow facility. Representative graph shows both anterior segments increase outflow facility after diazoxide treatment and return to baseline pressures after drug removal. Addition of glyburide inhibits the ability of diazoxide to increase outflow facility. DZ, diazoxide; Gly, glyburide.

FIGURE 4. Presence of K_{ATP} channel conductance in primary human NTM cells. Inside-out patch clamp shows increase of ionic conductance across membrane after diazoxide treatment as a function of membrane potential (mV). Channel openings are indicated by downward deflections. Glyburide inhibits ionic conductance associated with diazoxide treatment. Representative sweep steps from 0 mV to -100 mV are shown.

subunits are present in the TM, RT-PCR was performed on total RNA from TM and primary human NTM cells. In both TM and NTM cells, mRNA was found for K_{ir}6.1, K_{ir}6.2, SUR2A, and SUR2B (Fig. 5A). DNA sequence analysis confirmed that the amplified PCR product was the appropriate K_{ATP} channel subunit. SUR1 was not amplified in either TM or NTM cells. Using antibodies directed at the individual K_{ATP} subunits, IHC of TM and NTM cells showed expression of K_{ir}6.1, K_{ir}6.2, and SUR2B. SUR1 and SUR2A were not detected in TM tissue or in NTM cells.

Diazoxide and IOP In Vivo

To explore the feasibility of treating animals in vivo with K_{ATP} channel openers, we anesthetized 12 Brown Norway rats and

treated them unilaterally with a single dose of diazoxide. Within 1 hour of treatment, diazoxide decreased IOP in all 12 treated eyes compared with the fellow eye treated with vehicle alone (*P* < 0.04; Fig. 6). IOP consistently dropped in all treated eyes whereas vehicle raised IOP <5% at each time point. These results indicate that K_{ATP} channel activation by diazoxide decreases IOP in vivo.

DISCUSSION

Understanding the pathophysiology of outflow facility in normal and POAG eyes is essential for the identification of key molecules that can be used as therapeutic targets to lower IOP. We have identified K_{ATP} channel openers (diazoxide, nic-

FIGURE 5. K_{ATP} channel subunits in human TM and NTM cells. (A) Polymerase chain reaction confirmed the presence of all K_{ATP} channel subunits except SUR1. (B) IHC was performed with specific antibodies against human K_{ATP} channel subunits SUR1, SUR2A, SUR2B, K_{ir}6.1, and K_{ir}6.2. In TM tissue, uveal, corneal and juxtacanalicular cells stained positive for SUR2B (green) and K_{ir}6.1 (red). K_{ir}6.2 (red) showed a low level of expression in the TM. Primary human NTM cells stained positive for SUR2B (green), K_{ir}6.1, and K_{ir}6.2 (red). SUR1 and SUR2A were not identified in any of the tissues/cells analyzed by immunohistochemistry. Nuclei (blue) were stained with DAPI. SC, Schlemm's canal.

FIGURE 6. Addition of diazoxide to anesthetized rats decreases IOP. Diazoxide (5 mM) was added unilaterally to 12 anesthetized Brown Norway rats. IOP was assessed every 20 minutes over 4 hours. Each 1-hour data point is the average of three IOP measurements (20, 40, 60 minutes) taken with a rebound tonometer. * $P < 0.04$.

orandil, and P1075) as novel agents capable of increasing outflow facility in human anterior segment organ culture. Increased current flow and channel opening probability across membrane patches after K_{ATP} channel activation suggested the presence of functional K_{ATP} channels in the TM. The presence of K_{ATP} channel subunits ($K_{ir}6.1$, $K_{ir}6.2$, SUR2A, and SUR2B) was also verified by immunohistochemistry and RT-PCR. Furthermore, studies in Brown Norway rats confirmed that diazoxide can lower IOP in vivo. Taken together, these results indicate that K_{ATP} channels may be one of the molecular components involved in the regulation of outflow facility and IOP control.

POAG is a neuropathy caused by the loss of retinal ganglion cells within the optic nerve. An ideal therapeutic agent would be one that reduces IOP in the front of the eye and protects against neuronal damage in the back of the eye. Our studies suggest that K_{ATP} channel openers increase outflow facility by up to 90%, consistent with or even better than existing pressure-lowering agents such as latanoprost (67%).⁵⁸ In addition to increasing outflow facility, K_{ATP} channel openers are effective as retinal neuroprotective agents because opening of K_{ATP} channels with diazoxide provides ischemic preconditioning to retinas.^{23–25} K_{ATP} channel openers are already approved by the US Food and Drug Administration for the treatment of acute hypertension and may be considered future therapeutic agents for the treatment of ocular hypertension.

Identification of specific K_{ATP} channel subunit composition within the cells of the outflow pathways will be important for determining how these channels affect IOP. K_{ATP} channels that contain $K_{ir}6.1$ are predominantly found in vascular smooth muscle,²⁰ whereas $K_{ir}6.2$ containing channels are generally associated with nonvascular smooth muscle.¹⁹ In the TM, $K_{ir}6.1$ is present throughout the uveal, corneoscleral, and juxtacanalicular regions. $K_{ir}6.2$, however, is found at much lower levels throughout the tissue. This could indicate that there is a lack of $K_{ir}6.2$ in the TM and that the predominant inward rectifying subunit in K_{ATP} channels in the TM is $K_{ir}6.1$. Alternatively, it may represent different cell populations within the TM as the presence of at least two different cell populations have been reported.⁵⁹ Whether K_{ATP} channel combinations are different within these subpopulations of TM cells is yet to be determined. Because $K_{ir}6.1$ and $K_{ir}6.2$ channels have different conductance levels, future studies overexpressing specific K_{ATP} channel subunits in model systems such as *Xenopus laevis* oocytes and then comparing conductance levels in primary human NTM cells by patch clamp should identify specific subunit combinations. Furthermore, identifying the most influential K_{ATP} channel combination may have to be addressed in

$K_{ir}6.1^{-/-}$ or $K_{ir}6.2^{-/-}$ mice, which have already been used as viable mouse models in several cardioprotective studies.^{38,39,60,61}

SUR2A and SUR2B are generated from the same gene, *SUR2*, located on human chromosome 12p12.^{62–64} These two isoforms of the *SUR2* gene differ only in their 42 C-terminal amino acids. However, this 42 amino acid region has a critical role in K_{ATP} channel activation by influencing the binding of ATP and ADP.^{62,63} Both PCR and IHC indicate that SUR2B is present in the TM and human NTM cells, whereas the presence of SUR2A is less clear. Although PCR indicates the presence of SUR2A mRNA, IHC does not show SUR2A protein. Nevertheless, it appears that multiple K_{ATP} channel subunits are present in the TM. Further studies aimed at identifying the most influential K_{ATP} channel subunit combinations in the TM tissue will help in deciphering a key physiological function of these channels in the human eye.

Our study in Brown Norway rats indicates that diazoxide decreased IOP by ~15% within 2 hours of treatment, providing evidence that activation of K_{ATP} channels lowers IOP in vivo. Whether this effect by K_{ATP} channels is specific for the trabecular outflow pathway is unknown. Many tissues within the anterior chamber have K_{ATP} channel subunits (data not shown), indicating that K_{ATP} channels may function in those tissues as well. Given this information, it is possible that K_{ATP} channels across various tissues may work in conjunction to lower IOP through both the trabecular and uveoscleral pathways in vivo.

The physiological response of the outflow pathway that causes increased outflow facility ex vivo and decreased IOP in vivo after K_{ATP} channel activation is unknown. Recently, the calcium-activated potassium channel BK_{Ca} was shown to increase outflow facility after activation.⁷ The mechanism of action appears to be due to a decrease in TM cell volume caused by potassium ion efflux. Because K_{ATP} channels are weak inward rectifying channels, they are not one of the primary potassium channels directly involved in cell volume regulation. However, activation of K_{ATP} channels may influence cell shape by altering cellular contraction/relaxation, adhesion/cellular permeability, and adaptation to stress, all mechanisms associated with K_{ATP} channel activation in non-ocular cells and modulation of outflow facility in the eye.^{16,33–35,38–42,65,66} Furthermore, many of these processes have been associated with changes to the actin cytoskeleton. Interestingly, disruption of the actin cytoskeleton activates K_{ATP} channels through changes in ATP levels.^{67,68} It should also be noted that many channels, including K_{ATP} channels, do not act solely as individual entities but are part of a large macromolecular complex containing various kinases and cytoskeleton and adaptor proteins.^{69–71} Activation of K_{ATP} channels will influence these associated molecules, effectively altering downstream mechanisms. Understanding the role of K_{ATP} channel activation in IOP control will depend on the elucidation of the underlying molecular mechanisms and the physiological responses associated with K_{ATP} channel activation.

In summary, the presence of multiple K_{ATP} channel subunits within the TM in conjunction with the increase in outflow facility observed in anterior segment culture after treatment with K_{ATP} channel openers suggests that K_{ATP} channels have a prominent role in IOP regulation. Given the decrease of IOP observed in Brown Norway rats in vivo and the possibility that these channels might have an overall protective role on the outflow pathway, the use of K_{ATP} channel openers as IOP-lowering agents have the potential to become a future treatment modality for glaucoma.

References

- Lepple-Wienhues A, Rauch R, Clark AF, Grassmann A, Berweck S, Wiederholt M. Electrophysiological properties of cultured human trabecular meshwork cells. *Exp Eye Res*. 1994;59:305-311.
- Tamm E, Flugel C, Stefani FH, Rohen JW. Contractile cells in the human scleral spur. *Exp Eye Res*. 1992;54:531-543.
- Wiederholt M, Thieme H, Stumpff F. The regulation of trabecular meshwork and ciliary muscle contractility. *Prog Retin Eye Res*. 2000;19:271-295.
- O'Donnell ME, Brandt JD, Curry FR. Na-K-Cl cotransport regulates intracellular volume and monolayer permeability of trabecular meshwork cells. *Am J Physiol*. 1995;268:C1067-C1074.
- Tian B, Geiger B, Epstein DL, Kaufman PL. Cytoskeletal involvement in the regulation of aqueous humor outflow. *Invest Ophthalmol Vis Sci*. 2000;41:619-623.
- Wax MB, Tezel G, Kobayashi S, Hernandez MR. Responses of different cell lines from ocular tissues to elevated hydrostatic pressure. *Br J Ophthalmol*. 2000;84:423-428.
- Dismuke WM, Ellis DZ. Activation of the BK_{Ca} channel increases outflow facility and decreases trabecular meshwork cell volume. *J Ocul Pharmacol Ther*. 2009;4:309-313.
- Dismuke WM, Mbadugha CC, Ellis DZ. NO-induced regulation of human trabecular meshwork cell volume and aqueous humor outflow facility involve the BK_{Ca} ion channel. *Am J Physiol Cell Physiol*. 2008;294:C1378-C1386.
- Mitchell CH, Fleischhauer JC, Stamer WD, Peterson-Yantorno K, Civan MM. Human trabecular meshwork cell volume regulation. *Am J Physiol Cell Physiol*. 2002;283:C315-C326.
- Soto D, Comes N, Ferrer E, et al. Modulation of aqueous humor outflow by ionic mechanisms involved in trabecular meshwork cell volume regulation. *Invest Ophthalmol Vis Sci*. 2004;45:3650-3661.
- Liton PB, Gonzalez P. Stress response of the trabecular meshwork. *J Glaucoma*. 2008;17:378-385.
- Babenko AP, Aguilar-Bryan L, Bryan J. A view of sur/K_{IR}6.2, K_{ATP} channels. *Annu Rev Physiol*. 1998;60:667-687.
- Ashcroft FM, Harrison DE, Ashcroft SJ. Glucose induces closure of single potassium channels in isolated rat pancreatic beta-cells. *Nature*. 1984;312:446-448.
- Miki T, Liss B, Minami K, et al. ATP-sensitive K⁺ channels in the hypothalamus are essential for the maintenance of glucose homeostasis. *Nat Neurosci*. 2001;4:507-512.
- Suzuki M, Sasaki N, Miki T, et al. Role of sarcolemmal K(ATP) channels in cardioprotection against ischemia/reperfusion injury in mice. *J Clin Invest*. 2002;109:509-516.
- Zingman LV, Hodgson DM, Bast PH, et al. K_{ir}6.2 is required for adaptation to stress. *Proc Natl Acad Sci U S A*. 2002;99:13278-13283.
- Inagaki N, Tsuura Y, Namba N, et al. Cloning and functional characterization of a novel ATP-sensitive potassium channel ubiquitously expressed in rat tissues, including pancreatic islets, pituitary, skeletal muscle, and heart. *J Biol Chem*. 1995;270:5691-5694.
- Sakura H, Ammala C, Smith PA, Gribble FM, Ashcroft FM. Cloning and functional expression of the cDNA encoding a novel ATP-sensitive potassium channel subunit expressed in pancreatic beta-cells, brain, heart and skeletal muscle. *FEBS Lett*. 1995;377:338-344.
- Isomoto S, Kondo C, Yamada M, et al. A novel sulfonylurea receptor forms with BIR (K_{ir}6.2) a smooth muscle type ATP-sensitive K⁺ channel. *J Biol Chem*. 1996;271:24321-24324.
- Yamada M, Isomoto S, Matsumoto S, et al. Sulphonylurea receptor 2B and K_{ir}6.1 form a sulphonylurea-sensitive but ATP-insensitive K⁺ channel. *J Physiol*. 1997;499(pt 3):715-720.
- Inagaki N, Gonoi T, Clement JP, et al. A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K⁺ channels. *Neuron*. 1996;16:1011-1017.
- Lacza Z, Snipes JA, Miller AW, Szabo C, Grover G, Busija DW. Heart mitochondria contain functional ATP-dependent K⁺ channels. *J Mol Cell Cardiol*. 2003;35:1339-1347.
- Roth S, Dreixler JC, Shaikh AR, Lee KH, Bindokas V. Mitochondrial potassium ATP channels and retinal ischemic preconditioning. *Invest Ophthalmol Vis Sci*. 2006;47:2114-2124.
- Roth S, Li B, Rosenbaum PS, et al. Preconditioning provides complete protection against retinal ischemic injury in rats. *Invest Ophthalmol Vis Sci*. 1998;39:777-785.
- Roth S, Shaikh AR, Hennelly MM, Li Q, Bindokas V, Graham CE. Mitogen-activated protein kinases and retinal ischemia. *Invest Ophthalmol Vis Sci*. 2003;44:5383-5395.
- Singh H, Hudman D, Lawrence CL, Rainbow RD, Lodwick D, Norman RI. Distribution of K_{ir}6.0 and SUR2 ATP-sensitive potassium channel subunits in isolated ventricular myocytes. *J Mol Cell Cardiol*. 2003;35:445-459.
- Suzuki M, Kotake K, Fujikura K, et al. K_{ir}6.1: A possible subunit of ATP-sensitive K⁺ channels in mitochondria. *Biochem Biophys Res Commun*. 1997;241:693-697.
- Zhang C, Rosenbaum DM, Shaikh AR, et al. Ischemic preconditioning attenuates apoptotic cell death in the rat retina. *Invest Ophthalmol Vis Sci*. 2002;43:3059-3066.
- Zhou M, Tanaka O, Sekiguchi M, et al. Localization of the ATP-sensitive potassium channel subunit (K_{ir}6.1/uK(ATP)-1) in rat brain. *Brain Res Mol Brain Res*. 1999;74:15-25.
- Miura T, Miki T. ATP-sensitive K⁺ channel openers: old drugs with new clinical benefits for the heart. *Curr Vasc Pharmacol*. 2003;1:251-258.
- Group IS. Effect of nicorandil on coronary events in patients with stable angina: the Impact Of Nicorandil in Angina (IONA) randomised trial. *Lancet*. 2002;359:1269-1275.
- Wan Mohamad WB, Tun Fizi A, Ismail RB, Mafauzy M. Efficacy and safety of single versus multiple daily doses of glibenclamide in type 2 diabetes mellitus. *Diabetes Res Clin Pract*. 2000;49:93-99.
- Standen NB, Quayle JM, Davies NW, Brayden JE, Huang Y, Nelson MT. Hyperpolarizing vasodilators activate ATP-sensitive K⁺ channels in arterial smooth muscle. *Science*. 1989;245:177-180.
- Dal-Secco D, Cunha TM, Freitas A, et al. Hydrogen sulfide augments neutrophil migration through enhancement of adhesion molecule expression and prevention of CXCR2 internalization: Role of ATP-sensitive potassium channels. *J Immunol*. 2008;181:4287-4298.
- Kawamura T, Kadosaki M, Nara N, Wei J, Endo S, Inada K. Nicorandil attenuates NF- κ B activation, adhesion molecule expression, and cytokine production in patients with coronary artery bypass surgery. *Shock*. 2005;24:103-108.
- Brayden JE. Functional roles of K_{ATP} channels in vascular smooth muscle. *Clin Exp Pharmacol Physiol*. 2002;29:312-316.
- Daut J, Maier-Rudolph W, von Beckerath N, Mehrke G, Gunther K, Goedel-Meinen L. Hypoxic dilation of coronary arteries is mediated by ATP-sensitive potassium channels. *Science*. 1990;247:1341-1344.
- Kane GC, Behfar A, Dyer RB, et al. KCNJ11 gene knockout of the K_{ir}6.2 K_{ATP} channel causes maladaptive remodeling and heart failure in hypertension. *Hum Mol Genet*. 2006;15:2285-2297.
- Kane GC, Behfar A, Yamada S, et al. ATP-sensitive K⁺ channel knockout compromises the metabolic benefit of exercise training, resulting in cardiac deficits. *Diabetes*. 2004;53(suppl 3):S169-S175.
- Chatterjee S, Levitan I, Wei Z, Fisher AB. K_{ATP} channels are an important component of the shear-sensing mechanism in the pulmonary microvasculature. *Microcirculation*. 2006;13:633-644.
- Gao S, Long CL, Wang RH, Wang H. K(ATP) activation prevents progression of cardiac hypertrophy to failure induced by pressure overload via protecting endothelial function. *Cardiovasc Res*. 2009;83:444-456.
- Ozcan C, Terzic A, Bienengraeber M. Effective pharmacotherapy against oxidative injury: alternative utility of an ATP-sensitive potassium channel opener. *J Cardiovasc Pharmacol*. 2007;50:411-418.
- Alekseev AE, Hodgson DM, Karger AB, Park S, Zingman LV, Terzic A. ATP-sensitive K⁺ channel channel/enzyme multimer: metabolic gating in the heart. *J Mol Cell Cardiol*. 2005;38:895-905.
- Kane GC, Liu XK, Yamada S, Olson TM, Terzic A. Cardiac K_{ATP} channels in health and disease. *J Mol Cell Cardiol*. 2005;38:937-943.

45. Nichols CG, Lederer WJ. Adenosine triphosphate-sensitive potassium channels in the cardiovascular system. *Am J Physiol*. 1991; 261:H1675-H1686.
46. Seino S, Miki T. Physiological and pathophysiological roles of ATP-sensitive K⁺ channels. *Prog Biophys Mol Biol*. 2003;81:133-176.
47. Selivanov VA, Alekseev AE, Hodgson DM, Dzeja PP, Terzic A. Nucleotide-gated K_{ATP} channels integrated with creatine and adenylate kinases: amplification, tuning and sensing of energetic signals in the compartmentalized cellular environment. *Mol Cell Biochem*. 2004;256-257:243-256.
48. Zingman LV, Hodgson DM, Alekseev AE, Terzic A. Stress without distress: homeostatic role for K(ATP) channels. *Mol Psychiatry*. 2003;8:253-254.
49. Fautsch MP, Bahler CK, Jewison DJ, Johnson DH. Recombinant TIGR/MYOC increases outflow resistance in the human anterior segment. *Invest Ophthalmol Vis Sci*. 2000;41:4163-4168.
50. Fautsch MP, Bahler CK, Vrabel AM, et al. Perfusion of His-tagged eukaryotic myocilin increases outflow resistance in human anterior segments in the presence of aqueous humor. *Invest Ophthalmol Vis Sci*. 2006;47:213-221.
51. Johnson DH. The effect of cytochalasin D on outflow facility and the trabecular meshwork of the human eye in perfusion organ culture. *Invest Ophthalmol Vis Sci*. 1997;38:2790-2799.
52. Johnson DH, Tschumper RC. Human trabecular meshwork organ culture: a new method. *Invest Ophthalmol Vis Sci*. 1987;28:945-953.
53. Cao C, Healey S, Amaral A, et al. ATP-sensitive potassium channel: a novel target for protection against UV-induced human skin cell damage. *J Cell Physiol*. 2007;212:252-263.
54. Chi X, Sutton ET, Hellermann G, Price JM. Potassium channel openers prevent beta-amyloid toxicity in bovine vascular endothelial cells. *Neurosci Lett*. 2000;290:9-12.
55. de Klaver MJ, Buckingham MG, Rich GF. Isoflurane pretreatment has immediate and delayed protective effects against cytokine-induced injury in endothelial and vascular smooth muscle cells. *Anesthesiology*. 2003;99:896-903.
56. Kawano T, Zoga V, Gemes G, et al. Suppressed Ca²⁺/CaM/CaMKII-dependent K(ATP) channel activity in primary afferent neurons mediates hyperalgesia after axotomy. *Proc Natl Acad Sci U S A*. 2009;106:8725-8730.
57. Light PE, Manning Fox JE, Riedel MJ, Wheeler MB. Glucagon-like peptide-1 inhibits pancreatic ATP-sensitive potassium channels via a protein kinase A- and ADP-dependent mechanism. *Mol Endocrinol*. 2002;16:2135-2144.
58. Bahler C, Howell KG, Hann CR, Fautsch MP, Johnson DH. Prostaglandins increase trabecular meshwork outflow facility in cultured human anterior segments. *Am J Ophthalmol*. 2008;145:114-119.
59. Liton PB, Liu X, Stamer WD, Challa P, Epstein DL, Gonzalez P. Specific targeting of gene expression to a subset of human trabecular meshwork cells using the chitinase 3-like 1 promoter. *Invest Ophthalmol Vis Sci*. 2005;46:183-190.
60. Kane GC, Lam CF, O'Coilain F, et al. Gene knockout of the KCNJ8-encoded K_{ir}6.1 K(ATP) channel imparts fatal susceptibility to endotoxemia. *FASEB J*. 2006;20:2271-2280.
61. Minami K, Miki T, Kadowaki T, Seino S. Roles of ATP-sensitive K⁺ channels as metabolic sensors: studies of K_{ir}6.x null mice. *Diabetes*. 2004;53(suppl 3):S176-S180.
62. de Wet H, Fotinou C, Amad N, Dreger M, Ashcroft FM. The ATPase activities of sulfonylurea receptor 2A and sulfonylurea receptor 2B are influenced by the C-terminal 42 amino acids. *FEBS Lett J*. 2010;277:2654-2662.
63. Matsuoka T, Matsushita K, Katayama Y, et al. C-terminal tails of sulfonylurea receptors control ADP-induced activation and diazoxide modulation of ATP-sensitive K(+) channels. *Circ Res*. 2000;87:873-880.
64. Yamada M, Kurachi Y. A functional role of the C-terminal 42 amino acids of SUR2A and SUR2B in the physiology and pharmacology of cardiovascular ATP-sensitive K⁺ channels. *J Mol Cell Cardiol*. 2005;39:1-6.
65. Jons T, Wittschieber D, Beyer A, et al. K⁺-ATP-channel-related protein complexes: potential transducers in the regulation of epithelial tight junction permeability. *J Cell Sci*. 2006;119:3087-3097.
66. Rocheleau JV, Remedi MS, Granada B, et al. Critical role of gap junction coupled K_{ATP} channel activity for regulated insulin secretion. *PLoS Biol*. 2006;4:e26.
67. Furukawa T, Yamane T, Terai T, Katayama Y, Hiraoka M. Functional linkage of the cardiac ATP-sensitive K⁺ channel to the actin cytoskeleton. *Pfugers Arch*. 1996;431:504-512.
68. Terzic A, Kurachi Y. Actin microfilament disrupters enhance K_{ATP} channel opening in patches from guinea-pig cardiomyocytes. *J Physiol*. 1996;395-404.
69. Hund TJ, Mohler PJ. Differential roles for SUR subunits in K_{ATP} channel membrane targeting and regulation. *Am J Physiol Heart Circ Physiol*. 2011;300:H33-H35.
70. Kline CF, Kurata HT, Hund TJ, et al. Dual role of K_{ATP} channel C-terminal motif in membrane targeting and metabolic regulation. *Proc Natl Acad Sci U S A*. 2009;106:16669-16674.
71. Li J, Kline CF, Hund TJ, Anderson ME, Mohler PJ. Ankyrin-B regulates K_{ir}6.2 membrane expression and function in heart. *J Biol Chem*. 2010;285:28723-28730.