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ATP sensitive potassium channel openers: a new class of ocular hypotensive agents

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Abstract

ATP sensitive potassium (K_{ATP}) channels connect the metabolic and energetic state of cells due to their sensitivity to ATP and ADP concentrations. K_{ATP} channels have been identified in multiple tissues and organs of the body including heart, pancreas, vascular smooth muscles and skeletal muscles. These channels are obligatory hetero-octamers and contain four sulfonylurea (SUR) and four potassium inward rectifier (K_{ir}) subunits. Based on the particular type of SUR and K_{ir} present, there are several tissue specific subtypes of K_{ATP} channels, each with their own unique set of functions. Recently, K_{ATP} channels have been reported in human and mouse ocular tissues. In *ex vivo* and *in vivo* model systems, K_{ATP} channel openers showed significant ocular hypotensive properties with no appearance of toxic side effects. Additionally, when used in conjunction with known intraocular pressure lowering drugs, an additive effect on IOP reduction was observed. These K_{ATP} channel openers have also been reported to protect the retinal ganglion cells during ischemic stress and glutamate induced toxicity suggesting a neuroprotective property for this drug class. Medications that are currently used for treating ocular hypertensive diseases like glaucoma do not directly protect the affected retinal cells, are sometimes ineffective and may show significant side effects. In light of this, K_{ATP} channel openers with both ocular hypotensive and neuroprotective properties, have the potential to develop into a new class of glaucoma therapeutics.

Introduction

ATP sensitive potassium (K_{ATP}) channels are evolutionarily conserved membrane proteins that connect the metabolic and energetic state of cells by virtue of their sensitivity to micromolar concentrations of intracellular ATP and ADP.(Babenko et al., 1998) K_{ATP} channels have been reported in multiple organs and tissues of the body including heart, (Noma, 1983) pancreas, (Ashcroft et al., 1984; Cook and Hales, 1984) brain, (Ashcroft et

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al., 1984; Ashford et al., 1988) pituitary gland (Bernardi et al., 1993) and skeletal (Spruce et al., 1985) and smooth muscles. (Standen et al., 1989) K_{ATP} channels perform a multitude of functions but because of their sensitivity to ATP and ADP concentrations, these channels are often linked to changes in cellular metabolic states such as ischemia and hypoxia. (Rodrigo and Standen, 2005) One of the most well understood functions of K_{ATP} channels is in regulation of insulin secretion from the pancreatic beta cells. (Proks and Lippiat, 2006) In other organs such as heart and brain, the functions of K_{ATP} channels are mostly protective in nature. (Gross and Auchampach, 1992; Parratt and Kane, 1994; Rodrigo and Standen, 2005)

K_{ATP} channels have recently been identified in tissues of the anterior and posterior chambers of human and mouse eyes. (Chowdhury et al., 2013; Roy Chowdhury et al., 2015a) The presence of these channels and the effects of K_{ATP} channel openers and closers have suggested a role in intraocular pressure regulation. (Chowdhury et al., 2011; Chowdhury et al., 2013; Roy Chowdhury et al., 2015a) Additionally, K_{ATP} channels have been shown to be involved in protecting retinal cells in the presence of ischemic stress or glutamate induced toxicity. (Atlasz et al., 2007; Roth et al., 2006; Roth et al., 2003) In this review we summarize the salient features of K_{ATP} channels and highlight recent studies suggesting that pharmacologic openers of K_{ATP} channels such as diazoxide, nicorandil and cromakalim may be a new class of ocular hypotensive agents.

Molecular structure of K_{ATP} channels

K_{ATP} channels are transmembrane octameric proteins formed from two separate subunits, the regulatory sulfonylurea (SUR) subunit and the pore forming potassium inward rectifier (K_{ir}) subunit (Figure 1A). (Aguilar-Bryan et al., 1998; Aguilar-Bryan et al., 1995; Inagaki et al., 1995c) The SUR subunits are coded by two separate genes in humans – *SUR1* and *SUR2*. (Chutkow et al., 1996; Inagaki et al., 1995a) The initial SUR gene (*ABCC8*) was mapped to chromosome band 11p15.1 and its protein product (SUR1) was identified based on studies utilizing glibenclamide, a sulfonylurea compound that inhibited K_{ATP} channel activity. (Aguilar-Bryan et al., 1995) A second SUR gene (*ABCC9*) was identified at chromosome locus 12p12.1. (Thomas et al., 1995) The *ABCC9* gene gives rise to two separate subunits – SUR2A and SUR2B, through alternate splicing. (Chutkow et al., 1996) SUR2A and SUR2B are identical except for the terminal 42 amino acid residues (Figure 1B). (Aguilar-Bryan et al., 1998) Both SUR1 and SUR2 are members of the ATP binding cassette (ABC) transporter superfamily, a common membrane protein found in all species that use energy from ATP hydrolysis to selectively translocate specific molecules across the cell membrane. (Higgins and Linton, 2001; Tucker and Ashcroft, 1998) Similar to other ABC proteins, the SUR subunits contain two nuclear binding domains and two consensus nucleotide binding motifs. (Conti et al., 2001) Mutations in the *ABCC8* gene are known to cause hyperinsulinemic hypoglycemia in infants and have been linked to type II diabetes, (Laukkanen et al., 2004; Lohmueller et al., 2003; Meirhaeghe et al., 2001) while mutations in the *ABCC9* gene have been identified in sporadic cases of dilated cardiomyopathy, atrial fibrillation and hypertrichotic osteochondrodysplasia. (Bienengraeber et al., 2004; Harakalova et al., 2012; Olson et al., 2007; van Bon et al., 2012)

Initial studies with SUR1 and several known inward rectifiers (e.g. ROMK1, IRK1 etc.) (Ho et al., 1993; Makhina et al., 1994) failed to produce channel activity, (Aguilar-Bryan et al., 1995) suggesting that additional subunits were necessary to form functional K_{ATP} channels. This led to the cloning of *KCNJ8* (12p11.23) and *KCNJ11* (11p15.1), which code for $K_{ir}6.1$ and $K_{ir}6.2$. (Inagaki et al., 1996; Inagaki et al., 1995a; Inagaki et al., 1995b; Isomoto et al., 1996; Yamada et al., 1997) Mutations in the *KCNJ8 or KCNJ11* genes can lead to impaired cardiac adaptation to stress, heart disease (Gumina et al., 2007; Hodgson et al., 2003; Yamada et al., 2006) or an increased susceptibility to endotoxemia. (Kane et al., 2006) This has been found to be true in both mice and humans. (Hodgson et al., 2003; Kane et al., 2005)

The amino acid sequences of $K_{ir}6.1$ and $K_{ir}6.2$ have approximately 70% homology and share around 40–50% similarity with other K_{ir} channels. Like the SUR subunits, $K_{ir}6.1$ and $K_{ir}6.2$ failed to yield K^+ currents on their own. (Aguilar-Bryan et al., 1995; Inagaki et al., 1995a) However, combination of SUR and K_{ir} subunits forms active K_{ATP} channels. (Inagaki et al., 1996) SUR subunits contain nuclear binding domains and possess intrinsic ATPase activity making them the chief regulatory subunits that respond to intracellular metabolic changes. (Olson and Terzic, 2010) The SUR's nuclear binding domains can also attach to Mg-ADP, subsequently activating the subunits. Therefore, the hydrolysis of Mg-ATP by one of the nuclear binding domains can provide ADP for a separate nuclear binding domain to initiate channel activation, (Ueda et al., 1997; Ueda et al., 1999; Zingman et al., 2001) increasing the probability of maximum channel activation in the absence of ATP. (Babenko et al., 1999) The K_{ir} subunits can also bind with ATP but unlike SUR, they do so in the absence of Mg^{2+} and with much less affinity than that of a complete channel. (Tanabe et al., 2000; Tanabe et al., 1999) The SUR and K_{ir} subunits interact through various transmembrane domains. (Doyle et al., 1998; Kuo et al., 2003; Nishida and MacKinnon, 2002) Functionally, the K_{ir} subunit is involved in forming the channel pore while the SUR subunit is the main regulatory site, responsible for pharmacologic characteristics of the channels. (Aguilar-Bryan et al., 1995; Inagaki et al., 1995a; Sakura et al., 1995)

Localization and function

Depending on the particular SUR and K_{ir} subunits, there can be a total of 6 different subtypes of K_{ATP} channels. These subtypes are often tissue specific and can have characteristic functionalities of their own. (Rodrigo and Standen, 2005) In this section, we briefly describe the subunit composition and associated function of K_{ATP} channels in heart, muscles and pancreas where they have been studied in detail. For more information regarding K_{ATP} function in other tissues, readers may refer to the reviews by Rodrigo and Standen and Seino and Miki. (Rodrigo and Standen, 2005; Seino and Miki, 2003)

Cardiac tissue

In cardiac tissue, K_{ATP} channels are comprised of SUR2A and $K_{ir}6.2$ subunits. (Ashcroft and Ashcroft, 1990; Inagaki et al., 1996) These channels protect cardiac cells from Ca^{2+} overload, control cellular relaxation and are vital during ischemic episodes. (Noma, 1983; Ganote, 1983; Nichols and Lederer, 1991)

Vascular smooth muscle

In vascular smooth muscles, K_{ATP} channels are composed of SUR2B and $K_{ir}6.1$ subunits. These channels have less affinity to inhibition by intracellular ATP but respond strongly to nucleoside diphosphates, (Beech et al., 1993; Quayle et al., 1997) likely due to rather static ATP levels in vascular smooth muscle tissues except during extreme metabolic stress. (Rodrigo and Standen, 2005) As a result, ATP inhibition occurs at much lower levels in SUR2B and $K_{ir}6.1$ containing vascular K_{ATP} channels. The vascular K_{ATP} channels help in regulating blood flow by sensing various metabolic parameters like pH, adenosine levels and oxygen tension. Therefore it is not surprising that SUR2B and $K_{ir}6.1$ subunit containing channels play an important role in body homeostasis during periods of high metabolic activity like physical exercise. (Miki et al., 2002) A similar protective function during physical stress is also exhibited by $K_{ir}6.2$ subunit containing K_{ATP} channels of the heart. (Olson and Terzic, 2010; Zingman et al., 2002)

Skeletal muscle

One of the predominant roles of K_{ATP} channels in skeletal muscles is to increase vasodilation and formation of nitric oxide, (Marshall, 2000) particularly during fatigue. (Light et al., 1994) These channels are composed of SUR2A and $K_{ir}6.2$ (Allard and Lazdunski, 1993; Forestier et al., 1996) and their degree of inhibition by ATP is strongly regulated by changes in pH. (Davies et al., 1992) Similar to K_{ATP} channels present in other tissues, activation of these channels in skeletal muscles protects cells from excess Ca^{2+} accumulation, subsequently regulating contractile properties. (Burton and Smith, 1997; Matar et al., 2000; Seino and Miki, 2003) Additionally, studies in $K_{ir}6.2^{-/-)}$ mice indicate a regulatory role for K_{ATP} channels in cellular glucose absorption in skeletal muscles. (Miki et al., 1998)

Pancreas

K_{ATP} channels of the pancreas are composed of SUR1 and $K_{ir}6.2$ subunits (Inagaki et al., 1995a) and play a major role in regulating insulin secretion and subsequently glucose metabolism in the whole body. In pancreatic β cells, inhibition of K_{ATP} channels by ATP causes membrane depolarization that generates an associated voltage gated Ca^{2+} influx. Once the depolarization exceeds a cell specific threshold, (Ashcroft and Rorsman, 2013; Rorsman et al., 2011) insulin is released through exocytosis of intracellular granules. (Schulla et al., 2003) K_{ATP} channels have also been reported to affect glucagon secretion by α cells of the pancreas. (Gromada et al., 2004; Shiota et al., 2005) Both gain and loss of function mutations have been identified in the K_{ATP} channels of the pancreatic islets. Gain of function mutations in SUR1 or $K_{ir}6.2$ cause neonatal diabetes mellitus and loss of function mutations in these subunits cause congenital hyperinsulinism where insulin is constitutively secreted despite low levels of blood glucose. (Huopio et al., 2003; Ocal et al., 2011) K_{ATP} channel mutations have also been reported as causal for type 2 diabetes mellitus. (Gloyn et al., 2003; Sakura et al., 1995)

Pharmacologic openers and closers of K_{ATP} channels

Pharmacologic agents that affect the activation (opening) and inhibition (closing) of K_{ATP} channels have played an important role in understanding the functional significance of K_{ATP} channels. As such, many of these agents are used clinically to treat a multitude of disorders. K_{ATP} channel openers such as diazoxide, nicorandil and cromakalim have been used to treat hypertension, myocardial ischemia, bronchial asthma, urinary incontinence, hyperinsulinism, angina pectoris and some forms of skeletal muscle myopathies.(Hibino et al., 2010) In contrast, K_{ATP} channel closers stimulate insulin secretion and therefore pharmacologic closers like glibenclamide are one of the only oral medicines to treat diabetes. More recently, development of K_{ATP} channel closers called PNU compounds (PNU-37883A, a morpholinoguanidine and PNU-99963, a cyanoguanidine) have been shown to inhibit the vasodilation and hypotension caused by traditional K_{ATP} channel openers. (Khan et al., 1997; Meisheri et al., 1993)

In general, activation of K_{ATP} channels leads to membrane hyperpolarization whereas K_{ATP} channel closers cause depolarization of the cell membrane. However, K_{ATP} channel openers and closers are often specific for the subunit combination and hence have diverse roles based on the involved tissue. This specificity appears to be due to allosteric interactions involving the binding domains of the openers and closers with the nuclear binding domains of the SUR subunits. Diazoxide acts on SUR1 and SUR2B containing channels and can activate SUR2A channels only in the presence of MgADP. (Ashcroft and Gribble, 2000; Hibino et al., 2010; Isomoto et al., 1996; Yamada et al., 1997) Because of its specificity for SUR1 and SUR2B subunits, diazoxide is used to treat uncontrolled insulin secretion in conditions like persistent hyperinsulinemic hypoglycemia in infants. (Hibino et al., 2010) Nicorandil specifically activates SUR2B containing K_{ATP} channels and by virtue of its vasodilating actions through vascular smooth muscles, is used to treat angina without affecting the SUR1 containing pancreatic β cells. K_{ATP} channel closers glibenclamide and meglitinide block both SUR1 and SUR2 channels with equal efficacy whereas SUR1 selective blockers like tolbutamide have low affinity for SUR2 containing channels. (Gribble and Reimann, 2003; Hibino et al., 2010; Rodrigo and Standen, 2005)

Distribution of K_{ATP} channels and their electrophysiological properties in ocular cells and tissues

To evaluate the ocular hypotensive properties of K_{ATP} channel openers, it was essential to demonstrate the presence of functional K_{ATP} channels in the relevant ocular tissues. For this purpose we restricted our search to the trabecular meshwork and tissues of the anterior chamber as the primary regulatory units of the conventional outflow pathway. Patch clamp studies performed on isolated primary normal trabecular meshwork cells from human donors showed that channel opening probability at −60 mV increased from 0.29 ± 0.05 to 0.62 ± 0.05 in the presence of the K_{ATP} channel opener diazoxide (Figure 2). Additionally, the current magnitude increased from 1.68 ± 0.29 pA to 3.34 ± 0.35 pA. (Chowdhury et al., 2011) K_{ATP} channel activity was attenuated when glibenclamide (K_{ATP} channel closer) was

added to the cells, confirming the presence of functional K_{ATP} channels in normal trabecular meshwork cells (Figure 2).

Among the various subunits, SUR2A, SUR2B, $K_{ir}6.1$ and $K_{ir}6.2$ mRNAs were identified in human trabecular meshwork tissue as well as primary cultures of normal human trabecular meshwork cells but SUR1 was not detected. (Chowdhury et al., 2011) At the protein level, antibodies for the specific subunits identified SUR2B, $K_{ir}6.1$ and $K_{ir}6.2$ in human trabecular meshwork and Schlemm's canal tissue (Figure 3A–E). In mouse, tissues in the anterior chamber including the cornea, iris, ciliary body, trabecular meshwork and Schlemm's canal were all positive for SUR2B, $K_{ir}6.1$ and $K_{ir}6.2$ (Figure 3F–J). (Chowdhury et al., 2011; Chowdhury et al., 2013) Although SUR2A mRNA was detected, this subunit stained negative in immunohistochemistry of human and mouse ocular tissues (Figure 3B and G). The reason behind this discrepancy is unclear, but could be reasonably explained by the inability of the SUR2A commercial antibody to recognize the subunit or alternatively, SUR2A could be minimally expressed on cell membranes. Nevertheless, it is clear that multiple K_{ATP} channel subunits are present in various tissues in human and mouse eyes.

K_{ATP} channel openers as ocular hypotensive agents

Glaucoma is a progressive neurodegenerative disorder and the leading cause of irreversible blindness worldwide. (Quigley and Broman, 2006; Weinreb et al., 2014) By the year 2040 an estimated 110 million people will be affected by the disease. (Tham et al., 2014) Among various risk factors of glaucoma, elevated IOP is the only one that can be therapeutically modified. As a result, all treatment options for the disease – both pharmacological and surgical – are aimed at lowering IOP. (Heijl et al., 2002) Unfortunately, all current drugs used to treat glaucoma (e.g. prostaglandin analogs, carbonic anhydrase inhibitors, β blockers, etc.) are associated with side effects. (Roy Chowdhury et al., 2015b) Contraindicative symptoms include hypertrichosis, allergic conjunctivitis, hyperemia, blurred vision and even severe systemic cardiovascular episodes, particularly with β-blockers. (Aydin Kurna et al., 2014; Diggory and Franks, 1996; Johnstone, 1997; Mandell et al., 1988; Nguyen, 2014; Roy Chowdhury et al., 2015b; Wistrand et al., 1997) Additionally, no current therapeutics to treat glaucoma specifically targets the trabecular meshwork which is in part due to the incomplete understanding of the disease pathogenesis. (Weinreb et al., 2014; Weinreb and Khaw, 2004) Therefore, searching for novel drugs that can help treat the disease by addressing underlying physiologic events is of particular importance. (Crooke et al., 2012) Over the past five years, our laboratory has published several studies showing a novel ocular hypotensive property of K_{ATP} channel openers. (Chowdhury et al., 2011; Chowdhury et al., 2013; Roy Chowdhury et al., 2015a) In the following paragraphs, we review the experimental evidence and discuss the potentials and pitfalls of developing the K_{ATP} channel openers into a future therapeutic drug for treating glaucoma.

In non-ocular cells, the opening and closing of K_{ATP} channels modulates cellular contractility, cell adhesion, gap and tight junction regulation, adaptation to stress (shear, stretch, pressure, and oxidation), and improves overall cell well-being. (Brayden, 2002; Chatterjee et al., 2003; Dal-Secco et al., 2008; Gao et al., 2009; Kane et al., 2004; Kane et al., 2005; Kawamura et al., 2005; Nichols and Lederer, 1991; O'Donnell et al., 1995; Ozcan

et al., 2007; Seino and Miki, 2003; Standen et al., 1989; Zingman et al., 2003; Zingman et al., 2002) Interestingly, all these processes have been implicated in altering outflow facility in the eye and therefore in the pathophysiology of glaucoma. However, the role of K_{ATP} channels in ocular tissues has never been studied. We reasoned that in light of this functional relevance of K_{ATP} channels to the underlying events leading to glaucoma, these channels may also have a role in IOP regulation. To determine whether the opening of K_{ATP} channels had an effect on the trabecular meshwork and intraocular pressure regulation, we added K_{ATP} channel openers diazoxide, nicorandil and cromakalim to human anterior segment perfusion organ cultures. Diazoxide, nicorandil and cromakalim all showed reduction in pressure (diazoxide, 41%; nicorandil, 35%; cromakalim, 31%; pressure reduction compared to baseline) and outflow facility (80% with diazoxide; 50% for nicorandil; 50% with cromakalim) (Figure 4A–C). (Chowdhury et al., 2011; Chowdhury et al., 2013; Roy Chowdhury et al., 2015a). In comparison, latanoprost free acid, which is the active component of the glaucoma drug xalatan, increased outflow facility by 67% in this model. (Bahler et al., 2008) Pressure reduction with K_{ATP} channel openers was reversible and was completely inhibited by the K_{ATP} channel closer glibenclamide (Figure 4D).

For *in vivo* assessment, eye drops containing diazoxide or cromakalim were applied topically to one eye of wild type C57BL/6 mice once daily, while the contralateral eye received vehicle. Over a 14-day treatment period, diazoxide lowered IOP by 22% (maximal reduction of 25% on day 7) while cromakalim lowered IOP by 19% following a 5 day treatment period (maximal reduction of 23% on day 5) (Figure 5A and B), (Chowdhury et al., 2011; Chowdhury et al., 2013; Roy Chowdhury et al., 2015a) all slightly better than normotensive mice eyes treated with latanoprost, which produced an 18% IOP reduction when compared to control eyes. (Akaishi et al., 2009; Ota et al., 2005) Histologically, tissues of the conventional outflow pathway appeared normal in both human anterior segment cultures and in mouse eyes following treatment showing intact trabecular beams with viable trabecular meshwork cells and intact Schlemm's canal inner and outer walls with healthy appearing cells. This suggests that treatment with pharmacologic K_{ATP} channel openers appears to be safe since no toxic side effects were observed (Figure 5C and D). (Chowdhury et al., 2011; Chowdhury et al., 2013; Roy Chowdhury et al., 2015a)

Besides ocular hypotensive activity, the ability of a novel drug to work with existing glaucoma therapeutics to additively lower IOP would be a significant benefit in treating glaucoma. We have shown that cromakalim produces an additive IOP reduction when used in combination with the free acid form of latanoprost. For example, when C57BL/6 wild type mice were treated with both cromakalim and latanoprost, IOP was lowered by an additional 79% compared to cromakalim alone (Figure 6A) and 56% compared to latanoprost alone (Figure 6B). (Roy Chowdhury et al., 2015a) Ongoing studies are defining a novel signaling pathway distinct from latanoprost that is used by K_{ATP} channels to lower IOP (manuscript under preparation).

Subunit composition of K_{ATP} channels involved in lowering IOP

Since heterogeneity of K_{ATP} channels imparts selective specificity to the various pharmacologic openers, it is important to understand the particular subunit composition of

the K_{ATP} channels involved in lowering IOP. To determine subunit specificity, we obtained $K_{ir}6.2^{(-/-)}$ mice from Dr. Andre Terzic (Mayo Clinic). These mice are phenotypically normal, reproduce and live long lives similarly to wild-type controls. Other than a defective insulin secretion, these mice do not exhibit any major health issues under normal conditions. (Miki et al., 1998; Seino et al., 2000; Zingman et al., 2002) Only during cardiac adaptation in the event of physiologic stress (e.g. excessive physical exercise) do the animals encounter premature cardiac problems. (Zingman et al., 2003; Zingman et al., 2002) In the eye, $K_{ir}6.2^{(-/-)}$ mice looked normal at the levels of morphology and histology. The IOP in $K_{ir}6.2^{(-/-)}$ mice tends to be normal when compared to littermate controls indicating that the $K_{ir}6.2$ subunit is not required for pressure regulation under homeostatic conditions. (Chowdhury et al., 2013) Treatment of $K_{ir}6.2^{(-/-)}$ mice with diazoxide or cromakalim failed to lower IOP when compared to wild-type mice (Figure 5A and B). This indicates that $K_{ir}6.2$ subunit containing channels are essential for K_{ATP} channel mediated reduction of IOP. Since diazoxide and nicorandil act mainly on SUR2B containing K_{ATP} channels and have little or no effect on SUR2A channels, (Lawson, 2000; Yokoshiki et al., 1998; Vivaudou et al, 2010) we concluded that K_{ATP} channels containing both $K_{ir}6.2$ and SUR2B were the essential subunits required for IOP regulation.

K_{ATP} channels and neuroprotection of the retina

In addition to the ocular hypotensive effects of diazoxide, nicorandil and cromakalim, several reports have also indicated that K_{ATP} channel openers have a neuroprotective function in the eye. Opening of retinal K_{ATP} channels by diazoxide mimics ischemic preconditioning by activating downstream transduction of nitric oxide, particularly in the retinal ganglion cells. (Roth et al., 2006) Retinal ischemic preconditioning as seen by diazoxide treatment, can significantly decrease damage to the retina by inducing tolerance to ischemic events. (Roth et al., 1998) Diazoxide has also been shown to protect retinal degeneration caused by glutamate toxicity. (Atlasz et al., 2007) The neuroprotective effects of the K_{ATP} channel opener diazoxide is particularly interesting due to the fact that none of the current ocular hypotensive agents used for treating glaucoma has a direct cell protective effect on the retina.

Future direction with K_{ATP} channel openers

With excellent ocular hypotensive activity identified for K_{ATP} channel openers, the next logical step is to proceed to clinical trials. However, a significant roadblock to using K_{ATP} channel openers as a therapeutic for ocular hypertension is the insolubility of commercially available K_{ATP} channel openers in therapy friendly aqueous buffers. At present, diazoxide and cromakalim are soluble in dimethyl sulfoxide or other organic solvents which are not appropriate for topical application to human eyes. In addition, nicorandil has vasodilator activity attributed to a nitrate group in its chemical structure, (Nakae et al, 2000) making it a problematic compound to test the hypothesis that K_{ATP} channel openers lower IOP clinically in humans. In light of this, our laboratory has developed prodrugs based on the structure of the levo optical isomer of cromakalim. These novel prodrugs are aqueous soluble and on application to mouse eyes, are cleaved to generate the parent compound levcromakalim (unpublished data). The cromakalim prodrugs retain the IOP lowering ability of the parent

compound and results are encouraging, with several prodrugs showing similar IOP reduction as the parent compound in mouse and rabbit eyes (unpublished data). Additional studies are underway in our laboratory to understand the IOP lowering abilities of these prodrugs as well as their pharmacokinetic profiles. Once completed, we intend to move one of these compounds forward into phase 1 clinical trials.

Concluding remarks

K_{ATP} channels and their pharmacologic openers have important applications in a myriad of physiologic functions. K_{ATP} channel openers have been shown to lower IOP in several experimental models including *ex vivo* human anterior segment cultures and *in vivo* mouse models. Additionally, other laboratories have shown that K_{ATP} channel openers can protect retinal ganglion cells. Existing therapeutic strategies for treating glaucoma are often inadequate; come with unwanted side effects and none have direct neuroprotective properties, despite the fact that glaucoma is principally a disease of the retinal neurons. With the development of aqueous soluble prodrugs, K_{ATP} channel openers have a strong potential to become a new class of glaucoma therapeutics that can lower IOP and directly protect the retinal ganglion cells and the optic nerve from neuronal degeneration caused by elevated IOP.

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Abbreviations

K_{ATP} channels	ATP sensitive potassium channels
IOP	Intraocular pressure
SUR	Sulfonylurea
K_{ir}	Potassium inward rectifier

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Research Highlights

- ATP sensitive potassium channels are heteroctomeric containing sulfonylurea receptor (SUR) and potassium inward rectifying (K_{ir}) subunits.
- ATP sensitive potassium channels are found in many tissues of the body including the trabecular meshwork and retina.
- ATP sensitive potassium channel openers diazoxide, nicorandil and cromakalim show ocular hypotensive activity in *ex vivo* human anterior segment perfusion organ cultures and *in vivo* in mice.
- ATP sensitive potassium channels have direct neuroprotective properties on retinal ganglion cells.

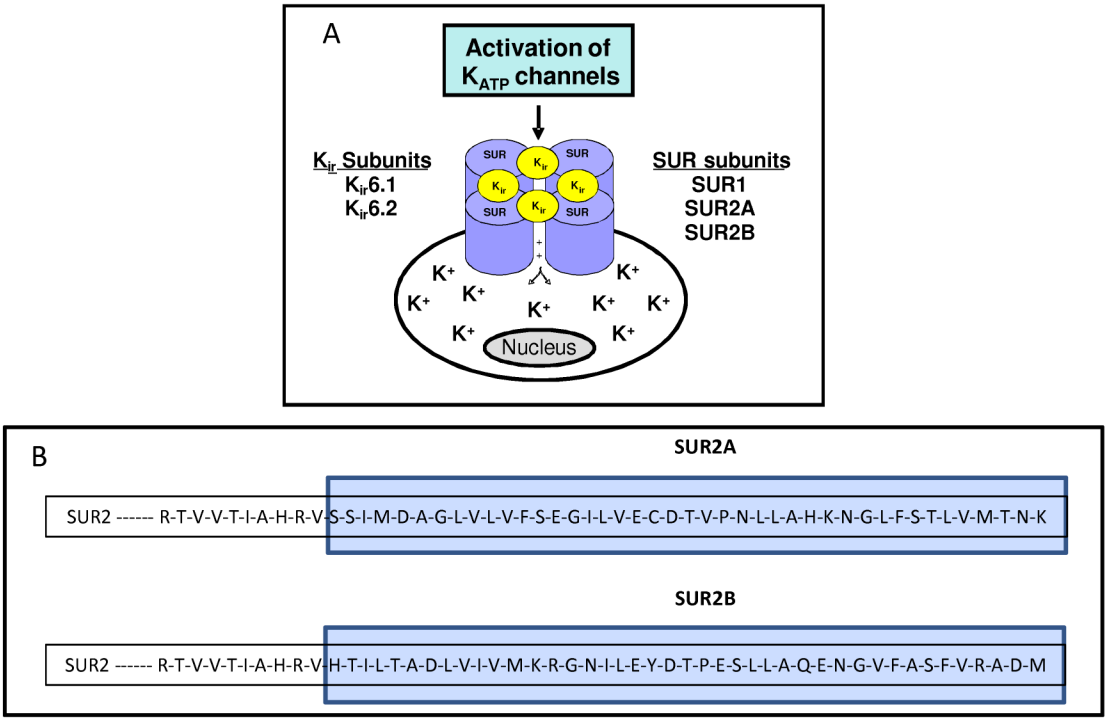

Figure 1. Molecular structure of K_{ATP} channels

(A) Association of the sulfonylurea receptor (SUR) and the potassium inward rectifying (K_{ir}) subunits in a functional K_{ATP} channel. The SURs form the regulatory subunit and are named due to their affinity for sulfonylureas which block the channel. The K_{ir} subunits form the pore forming unit which allows selective translocation of K^+ ions based on metabolic states of the cell. Depending on the particular SUR and K_{ir} subunit combination, there can be six different sub-types of K_{ATP} channels. These subtypes are tissue specific and have varied specificity towards pharmacologic openers and closers of these channels. (B) SUR2A and SUR2B are both translated from the SUR2 gene and have 99% homology. The two subunits differ only in the last 42 amino acid residues (blue box) in the C-terminus. This is due to alternative splicing of the two 3′ terminal exons of the SUR2 gene.

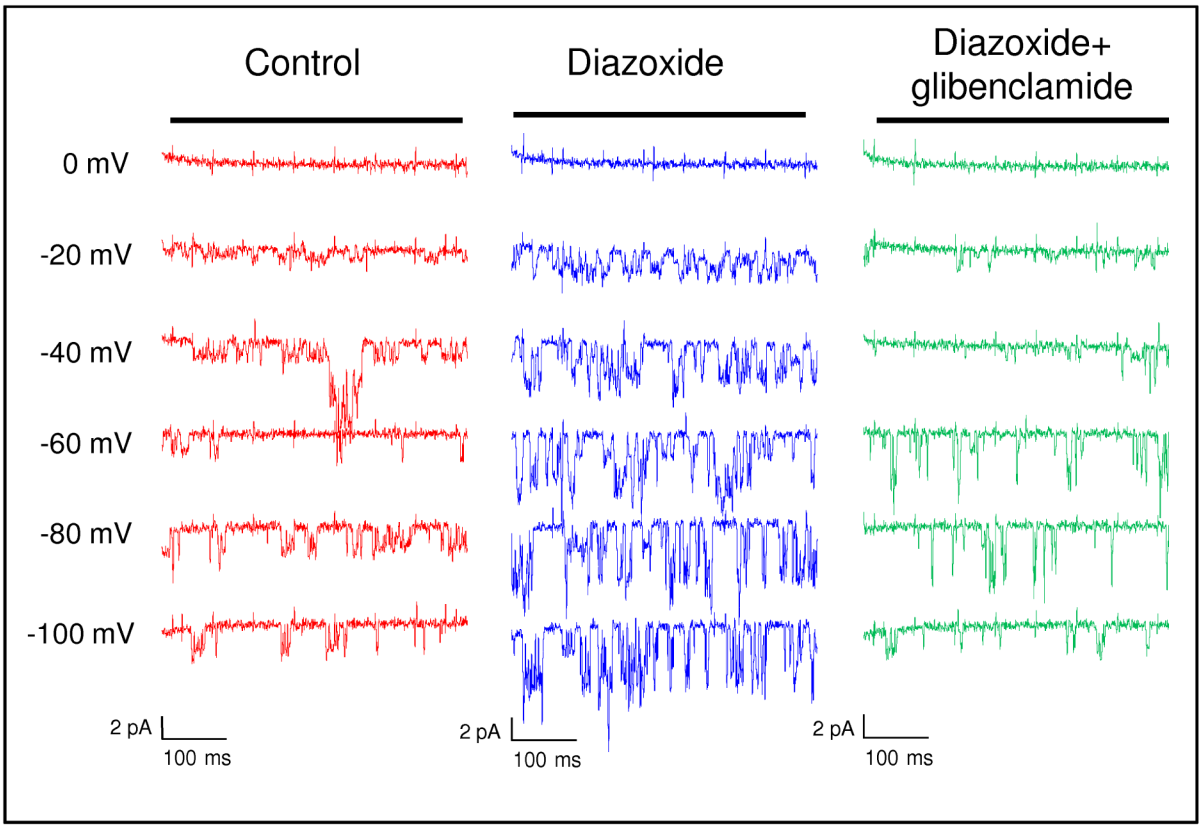

Figure 2. K_{ATP} channel activity in primary trabecular meshwork cells

Patch clamp recordings show increased K^+ conductance across the cell membranes of normal trabecular meshwork primary cells following diazoxide treatment. Channel opening probability in diazoxide treated cells was also increased at −60 mV as indicated by downward deflections. Effect of diazoxide was inhibited by the K_{ATP} channel closer glibenclamide. Figure shows representative sweep steps from 0 mV to −100 mV. Figure was previously published in Chowdhury et al., 2011.

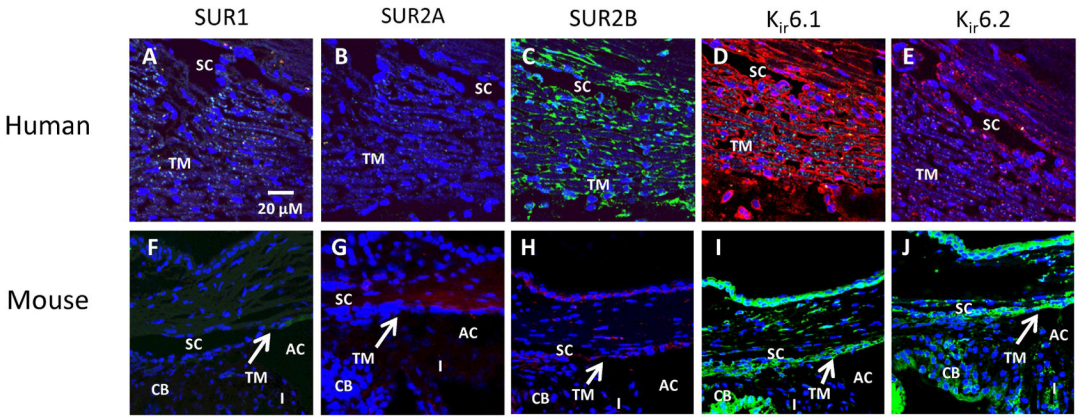

Figure 3. Immunohistochemical localization of K_{ATP} channel subunits in tissues of the conventional outflow pathway

(A–E) In normal human trabecular meshwork tissue, SUR2B, $K_{ir}6.1$ and $K_{ir}6.2$ were present while SUR1 and SUR2A were absent. (F–J) In mouse eyes, results similar to human studies were obtained – SUR2B, $K_{ir}6.1$ and $K_{ir}6.2$ were present while SUR1 and SUR2A were absent. Some auto-fluorescence was observed with SUR1 and SUR2A antibodies but the overall intensity was much lower than the subunits that stained positive. SUR2B, $K_{ir}6.1$ and $K_{ir}6.2$ were evenly distributed in the trabecular meshwork (TM), ciliary body (CB), iris (I) and inner and outer wall of Schlemm's canal (SC). AC, anterior chamber. Figure reproduced from images published in Chowdhury et al., 2011 and Chowdhury et al., 2013.

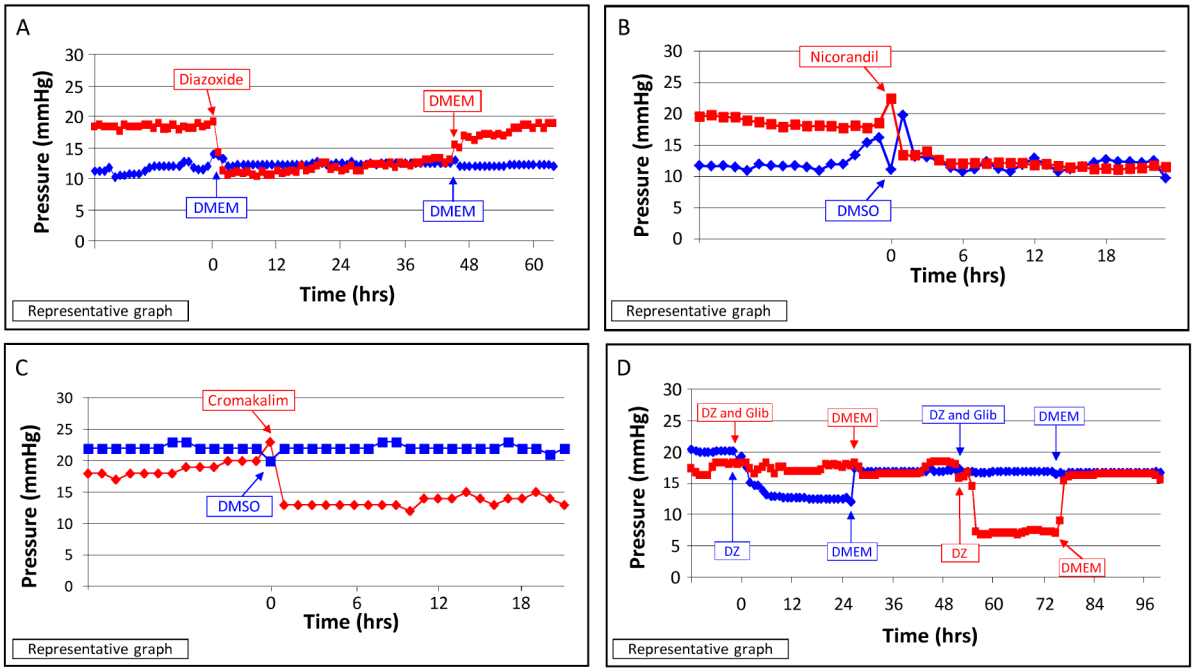

Figure 4. Effect of K_{ATP} channel openers on pressure in human anterior segment perfusion culture

(A) Diazoxide, (B) nicorandil, and (C) cromakalim significantly lowered pressure in human anterior segment perfusion cultures. (D) Addition of glibenclamide (Glib) inhibited the pressure lowering effect of diazoxide (DZ). DMSO, dimethyl sulfoxide; DMEM, Dulbecco's Modified Eagle's Media. Images reproduced from figures published in Chowdhury et al., 2011 and Roy Chowdhury et al, 2015a.

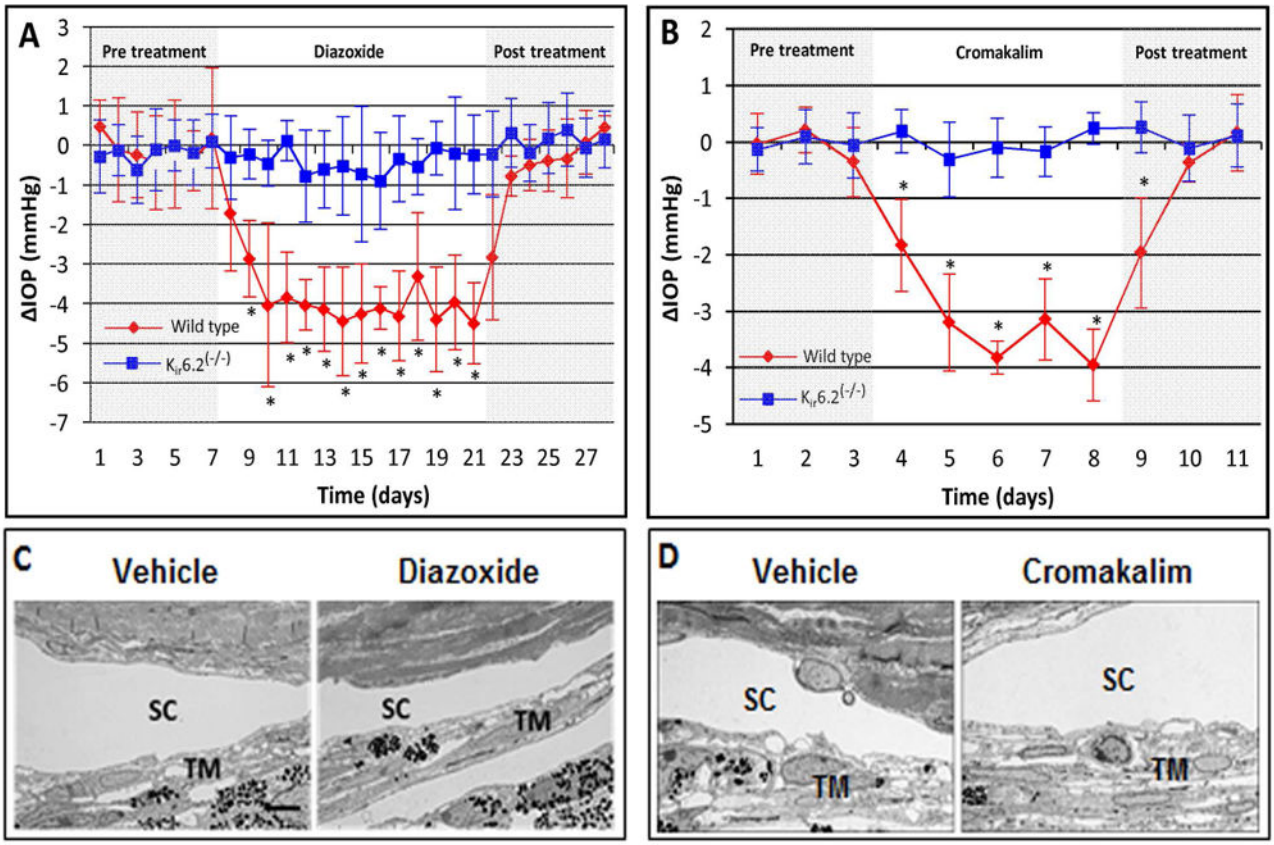

Figure 5. K_{ATP} channel openers significantly lower IOP in wild type C57BL/6 mice

(A) Diazoxide lowered IOP in wild type C57BL/6 mice (red diamonds). However, diazoxide did not lower IOP in $K_{ir}6.2^{(-/-)}$ mice (blue squares). (B) Cromakalim lowered IOP in wild type mice and similar to diazoxide (red diamonds), failed to lower IOP in $K_{ir}6.2^{(-/-)}$ mice (blue squares). (C) Histology of cells and tissues of the conventional outflow pathway in wild type C57BL/6 mice following diazoxide treatment. (D) Histology of cells and tissues of the conventional outflow pathway in wild type C57BL/6 mice following cromakalim treatment. Neither diazoxide nor cromakalim showed toxic side effects in conventional outflow tissues following treatment. SC, Schlemm's canal; TM, trabecular meshwork. Scale bar in (C) represents 5 μm for images (C) and (D). Average baseline IOP for wild type and $K_{ir}6.2^{(-/-)}$ mice was 15.9 ± 0.6 and 16.5 ± 1.1 mm Hg respectively. *p<0.05. Figures reproduced from images published in Chowdhury et al., 2013 and Roy Chowdhury et al., 2015a.

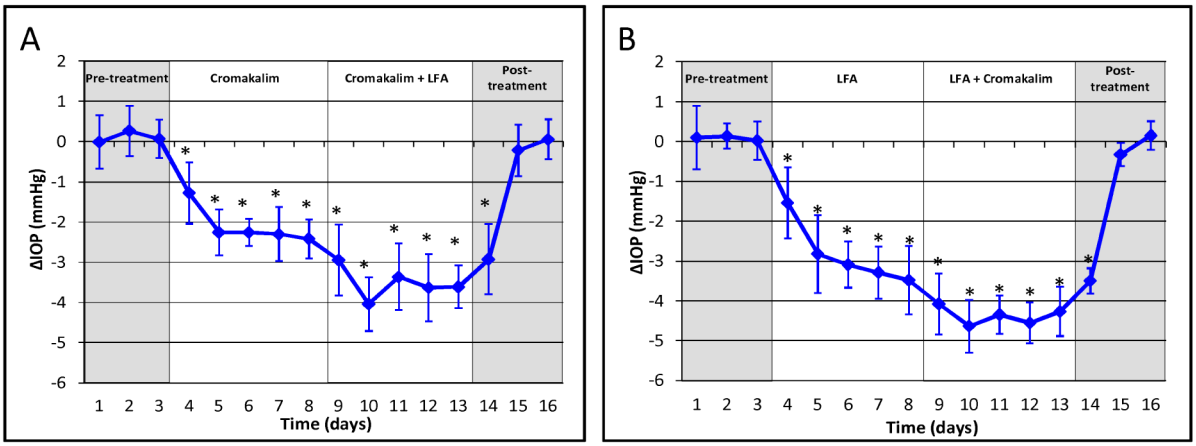

Figure 6. Combination treatment with K_{ATP} channel openers and latanoprost free acid (LFA)

(A) Cromakalim and LFA when used in combination therapy in C57BL/6 wild type mice reduced IOP by 79% more than cromakalim alone. (B) Combination treatment of cromakalim and LFA lowered IOP by 56% more than treatment with latanoprost alone. Average baseline IOP for wild-type mice was 16.2 ± 0.3 mm Hg. Figures reproduced from Roy Chowdhury et al., 2015a.