



Published in final edited form as:

*Sheng Li Xue Bao*. 2012 February 25; 64(1): 1–13.

## **K<sub>ATP</sub> channel action in vascular tone regulation: from genetics to diseases**

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### **Abstract**

ATP-sensitive potassium (K<sub>ATP</sub>) channels are widely distributed in vasculatures, and play an important role in the vascular tone regulation. The K<sub>ATP</sub> channels consist of 4 pore-forming inward rectifier K<sup>+</sup> channel (Kir) subunits and 4 regulatory sulfonylurea receptors (SUR). The major vascular isoform of K<sub>ATP</sub> channels is composed of Kir6.1/SUR2B, although low levels of other subunits are also present in vascular beds. The observation from transgenic mice and humans carrying Kir6.1/SUR2B channel mutations strongly supports that normal activity of the Kir6.1/SUR2B channel is critical for cardiovascular function. The Kir6.1/SUR2B channel is regulated by intracellular ATP and ADP. The channel is a common target of several vasodilators and vasoconstrictors. Endogenous vasopressors such as arginine vasopressin and  $\alpha$ -adrenoceptor agonists stimulate protein kinase C (PKC) and inhibit the K<sub>ATP</sub> channels, while vasodilators such as  $\beta$ -adrenoceptor agonists and vasoactive intestinal polypeptide increase K<sub>ATP</sub> channel activity by activating the adenylate cyclase-cAMP-protein kinase A (PKA) pathway. PKC phosphorylates a cluster of 4 serine residues at C-terminus of Kir6.1, whereas PKA acts on Ser1387 in the nucleotide binding domain 2 of SUR2B. The Kir6.1/SUR2B channel is also inhibited by oxidants including reactive oxygen species allowing vascular regulation in oxidative stress. The molecular basis underlying such a channel inhibition is likely to be mediated by S-glutathionylation at a few cysteine residues, especially Cys176, in Kir6.1. Furthermore, the channel activity is augmented in endotoxemia or septic shock, as a result of the upregulation of Kir6.1/SUR2B expression. Activation of the nuclear factor- $\kappa$ B dependent transcriptional mechanism contributes to the Kir6.1/SUR2B channel upregulation by lipopolysaccharides and perhaps other toll-like receptor ligands as well. In this review, we summarize the vascular K<sub>ATP</sub> channel regulation under

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physiological and pathophysiological conditions, and discuss the importance of  $K_{ATP}$  channel as a potentially useful target in the treatment and prevention of cardiovascular diseases.

## Keywords

ATP-sensitive potassium channel; Kir6.1; SUR2B; protein phosphorylation; S-glutathionylation; nuclear factor- $\kappa$ B; sepsis; sudden infant death syndrome; J-wave syndrome

## 1 Introduction

The ATP-sensitive  $K^+$  ( $K_{ATP}$ ) channel was firstly identified by Noma in cardiac myocytes [1]. The  $K_{ATP}$  channel differs from other inward rectifier  $K^+$  channels (Kir) in that it is sensitive to intracellular ATP concentration [1]. Besides in cardiac myocytes,  $K_{ATP}$  channels have also been found in skeletal myocytes [2] and pancreatic  $\beta$  cells [3]. In 1989, Standen *et al.* [4] recorded a novel  $K_{ATP}$  current in rabbit mesenteric arterial smooth muscle cells (SMCs). The current is enhanced by vasoactive intestinal polypeptide (VIP) and a  $K_{ATP}$  channel opener cromakalim, and suppressed by glibenclamide, similar to currents carried by other  $K_{ATP}$  channels identified previously. In the presence of pinacidil, the Kir6.1/SUR2B is stimulated by micromolar ATP and millimolar UDP or ADP, and higher doses (1–3 mmol/L) of ATP inhibits the channel, properties that lead to the name  $K_{NDP}$  channel [5]. Subsequently,  $K_{ATP}$  channels were found to be broadly expressed in vasculatures [6, 7]. Cloning of Kir6.x and SUR subunits of  $K_{ATP}$  channels in the mid-1990s, and the generation of  $K_{ATP}$  channel transgenic mice in 2000s, have greatly extend our understanding in the biophysical features and physiological functions of vascular  $K_{ATP}$  channel. Recently, reports of  $K_{ATP}$  mutations in human patients start to reveal the role of  $K_{ATP}$  channel in pathophysiological conditions [8–10].

## 2 Molecular structures of vascular $K_{ATP}$ channels

$K_{ATP}$  channels are octameric protein complexes containing 4 pore-forming Kir6 subunits and 4 accessory sulfonylurea receptor (SUR) subunits (Fig. 1). To date, two Kir6.x genes (*KCNJ8* for Kir6.1, and *KCNJ11* for Kir6.2) and two SUR genes (*ABCC8* for SUR1 and *ABCC9* for SUR2A and SUR2B) have been identified. The Kir6.x share 40%–50% homology in amino acid sequence with other Kir channels. Structural studies suggest the Kir6.x subunit has 2 transmembrane helices (M1 and M2), cytoplasmic N- and C-termini and a pore-forming loop with a glycine-phenylalanine-glycine signature motif for  $K^+$  selectivity [11]. In symmetrical 140 mmol/L  $K^+$  recording conditions, the unitary conductance of Kir6.1-containing channels is ~35 pS (Kir6.1/SUR2B) [12], whereas Kir6.2-containing channels is ~80 pS (Kir6.2/SUR2B) [13].

Functional expression of  $K_{ATP}$  channel requires co-expression of SUR subunit [14], which is under the category of ATP-binding cassette transporter (ABCC) family. SUR1 is dominantly expressed in pancreatic  $\beta$  cells. SUR2 has two variants: SUR2A and SUR2B, which are produced by alternative splicing of exon 38 in *ABCC9* [13, 15]. They are different in the last 42 amino acids in the C terminus. SUR2A is mainly expressed in myocardium and skeletal muscles, whereas SUR2B is generally distributed in smooth muscles. SUR subunit has 3

transmembrane domains (Fig. 1): TMD1 and TMD2 with 6 transmembrane segments in each, plus TMD0, an N-terminal transmembrane domain with 5 transmembrane segments. There are two large intracellular loops connecting the adjacent TMDs (Fig. 1). Each intracellular loop contains a nucleotide binding domain (NBD1 and NBD2, respectively). A Walker A motif ( $W_A$ ), a Walker B motif ( $W_B$ ), and a linker region are located within the NBDs and are critical for nucleotide binding [11].

The main body of SUR2B (TMD1, 2 and NBD1, 2) shares many properties with other transmembrane AB-CCs. Thereof it can be modeled with the bacteria ATP binding protein SAV1866 [16]. The TMDs and NBDs display a twisted interaction: TMD1 mainly interacts with NBD2 and TMD2 mainly with NBD1 [16]. This intercrossed interaction might have a significant effect on protein kinase A (PKA) phosphorylation and regulation, for a phosphorylation site has been found in NBD2 domain (see below for detail).

### 3 Evidence of vascular $K_{ATP}$ channel function from transgenic mouse models and human diseases

$K_{ATP}$  channels have been demonstrated to play a substantial role in vascular tone regulation by using traditional pharmacological approaches, including the application of  $K_{ATP}$  channel openers, such as pinacidil, diazoxide and cromakalim, and  $K_{ATP}$  channel blockers, such as sulfonylureas (tolbutamide, glibenclamide) and PNU-37883A. Transgenic mouse models provide more specific strategies to understand the impacts of  $K_{ATP}$  channels on cardiovascular system. In general, both Kir6.1 and SUR2 knockout mice exhibit coronary arterial spasm and sudden early death, with EKG showing frequently spontaneous ST segment elevation [17, 18]. In addition, Kir6.1-null mice are more sensitive to endotoxemia, suggesting functional  $K_{ATP}$  channel is important for survival from sepsis [19, 20]. Since  $K_{ATP}$  channel is distributed in vascular endothelial cells as well as SMCs, both of which contribute to vascular tone regulation, the function of endothelial  $K_{ATP}$  channel has been noticed recently. In a transgenic animal model, SUR2B expression is selectively *knocked in* in SMCs. These SUR2-null mice remain to show coronary vasospasm similar to Kir6.1 knockout mice, suggesting that  $K_{ATP}$  channel in vascular smooth muscle (VSM) is not enough for vascular tone regulation [21]. In another study, transgenic mice expressing dominant negative Kir6.1 subunits exclusively in endothelium exhibit an elevated endothelin-1 (ET-1) release and an increase in coronary resistance [22]. These observations thus suggest that endothelial  $K_{ATP}$  channel is important for coronary circulation.

Recently, several mutations of vascular  $K_{ATP}$  channels were found in human patients. A missense mutation in exon 3 (S422L) of *KCNJ8* was identified in a patient presenting massive accentuation of the early repolarization and recurrent ventricular fibrillation in EKG with normal coronary angiography [8]. The S422L mutation was also found by another group in 2 patients presenting J-wave syndrome [9]. The current density of Kir6.1 S422L/SUR2A channel heterologously expressed in COS-1 cells is increased by ~65%. Two other *KCNJ8* mutations (an in-frame deletion E332del and a missense mutation V346I) located at Kir6.1's C-terminus were found in sudden infant death syndrome (SIDS) patients. Patch clamping shows that the pinacidil-stimulated  $K_{ATP}$  currents are reduced by ~ 50% in

E332del and V346I. The loss-of-function *KCNJ8* mutations may result in a maladaptive cardiac response to systemic metabolic stimulators leading to SIDS<sup>[10]</sup>.

## 4 Sepsis susceptibility

In a genome-wide association study using *N*-ethyl-*N*-nitrosourea *in-vivo* mutagenesis, Beutler and his colleagues screened a large population of mice and identified 4 strains that were highly susceptible to multiple infectious pathogens, including cytomegalovirus, lipopolysaccharides (LPS), synthetic Toll-like receptor 3 (TLR3) ligand polyinosine: polycytidylic acid and TLR9 agonists CpG oligodeoxynucleotides<sup>[19]</sup>. They have found that the high sepsis susceptibility is due to *Kcnj8*, as disruptions of the locus containing *Kcnj8* are present in the homozygous form in all the 4 strains of mice. Their mutagenesis study suggests that the LPS hypersensitivity phenotype is not suppressed by mutations in *Myd88*, *Trif*, *Tnf*, *Tnfrsf1a*, *Ifnb*, *Ifng* or *Stat1* as well as several other genes known to contribute to inflammation responses. The investigators believe that their forward genetic approaches also can exclude tumor necrosis factor (TNF), type-I interferon (IFN), and type-II IFN as essential lethal factors, because mice that lacked these receptor genes succumbed to low doses of LPS. These control studies strongly suggest that the sepsis hypersusceptibility is not a result of these genes and pro-inflammation cytokines. Consistent with these observations, *Kcnj8*-knockout mice show severe survival disadvantages in response to septic pathogens, with progressive deterioration in cardiac activity, ischemic myocardial damage, and myocardial contractile dysfunction<sup>[20]</sup>. Since genetic disruption of  $K_{ATP}$  channels is not lethal in mice, these studies indicate that activation of the  $K_{ATP}$  channels is crucial for the systemic response to sepsis by retaining myocardial perfusion.

Studies by Beutler, Hoffman and their colleagues indicate that the  $K_{ATP}$  channel also functions in antiviral activity in *Drosophila*. Knockout of the *dSUR* gene increases the lethality of *Drosophila* after infection with the cardiotrophic flock house virus (FHV)<sup>[19]</sup>. Similar effects were observed by knockdown of both *Ir* and *Ir2* genes<sup>[23]</sup>. In the  $K_{ATP}$  mutant flies, FHV causes rapid viremia and death, likely to be mediated by modulating the antiviral RNA interference in the heart, while flies treated with the  $K_{ATP}$  agonist pinacidil are protected against the viral infection<sup>[23]</sup>.

## 5 Regulation of vascular $K_{ATP}$ channels

The regulation of vascular  $K_{ATP}$  channels is composed by an immediate and a delayed phase. The immediate regulation by most of metabolites, hormones and neurotransmitters is through channel gating, whereas transcriptional mechanisms contribute to the delayed regulation.

### 5.1 Metabolites

**5.1.1 ATP/ADP—** $K_{ATP}$  channels are subject to a direct and fast regulation by intracellular ATP and ADP. Such modulations directly link the cellular metabolic states to membrane electric activities. ATP reduces the vascular  $K_{ATP}$  channel activity; however, the inhibitory effects of ATP are variable in different reports<sup>[24]</sup>. Due to a relatively high intracellular ATP concentration in physiological condition (1–11.7 mmol/L), the vascular  $K_{ATP}$  channels

usually display a low activity at rest<sup>[25]</sup>. In comparison, intracellular ADP concentration ranges between 0.1 and 3 mmol/L <sup>[25]</sup>, and exhibits stimulatory effect on K<sub>ATP</sub> channel. According to this characteristic, vascular K<sub>ATP</sub> channel was once termed as K<sub>NDP</sub> channel<sup>[5, 26]</sup>.

**5.1.2 pH**—pH changes in local tissues are very common in heavy exercise, hypoxia, ischemia, and severe diabetes. Hypercapnia and acidosis relax blood vessels, especially cerebral arterioles, and increase regional blood flow in circulation<sup>[27, 28]</sup>. Hypercapnic acidosis induces vasodilation through activation of K<sub>ATP</sub> channels in VSMs, with maximal effect at pH 6.5 to 6.8 <sup>[29]</sup>. Blockade of K<sub>ATP</sub> channels attenuates the vasodilation, which is observed in cerebral arterioles, basil artery, coronary artery, mesenteric artery or internal mammary artery. The modulations of K<sub>ATP</sub> channel activity by H<sup>+</sup>, ATP and ADP are mediated via direct ligand binding to Kir6.x or SUR, leading to alternation in the channel gating <sup>[30–32]</sup>.

**5.1.3 Nitric oxide (NO)**—NO is released by endothelial cells and causes vasodilation. It is reported NO hyperpolarizes SMCs in rabbit mesenteric arteries through increasing cGMP and activating K<sub>ATP</sub> channels<sup>[33]</sup>. NO released from skeletal muscle vasculatures during excise may activate vascular K<sub>ATP</sub> channels, and antagonizes sympathetic vaso-constriction, providing a delicate mechanism to regulate blood flow in exercising skeletal muscles<sup>[34]</sup>. Lactate, an important metabolic product in retina, relaxes retinal arterioles through activation of nitric oxide synthase (NOS) and guanylyl cyclase, and opening of K<sub>ATP</sub> channel <sup>[35]</sup>. However, it is also reported that NO donor sodium nitroprusside fails to activate K<sub>ATP</sub> currents isolated from rabbit mesenteric arterial SMCs and pig coronary arterial SMCs <sup>[36, 37]</sup>. Therefore, the exact role of NO in regulating vascular K<sub>ATP</sub> channels is still debatable.

**5.1.4 Eicosanoids**—Epoxyeicosatrienoic acids (EETs) are cytochrome P-450 metabolites of arachidonic acid synthesized in endothelial cells<sup>[38]</sup>. Since EETs participate in vasodilation by hyperpolarizing cell membrane, some groups classified them in endothelium-derived hyperpolarizing factors (EDHFs) <sup>[39, 40]</sup>. Both 11, 12-EET and 14, 15-EET induce dose-dependent vasodilation in isolated small mesenteric arteries through activation of K<sub>ATP</sub> channels <sup>[41, 42]</sup>, but the underlying mechanisms seem to be different: 11, 12-EET activates mesenteric SMC K<sub>ATP</sub> channels through PKA <sup>[42]</sup>, whereas the stimulation of 14, 15-EET depends on ADP-ribosylation of G<sub>s</sub> <sup>[41]</sup>.

**5.1.5 Hydrogen sulfide (H<sub>2</sub>S)**—H<sub>2</sub>S is a product from *L*-cysteine metabolism catalyzed by cystathionine-γ-lyase and cystathionine-β-synthase in mammalian tissues. Endogenous H<sub>2</sub>S has been detected in various vascular tissues (e.g. aorta, tail, and mesenteric arteries) <sup>[43]</sup>. H<sub>2</sub>S in physiological concentrations (nearly 45 μmol/L) induces vasodilation in rat aorta and transient reduction of blood pressure through activation of K<sub>ATP</sub> channels <sup>[44, 45]</sup>. Pinacidil- and H<sub>2</sub>S-induced vasorelaxation are compromised in cerebral arterioles of SUR2-null mice<sup>[46]</sup>. Patch clamping studies demonstrate that exogenous H<sub>2</sub>S activates K<sub>ATP</sub> channels and hyperpolarizes cell membrane in SMC isolated from rat mesenteric artery <sup>[47]</sup> and piglet cerebral arterioles <sup>[46]</sup>. In addition, aortic rings seem to be

more sensitive to H<sub>2</sub>S than pulmonary arterial rings. The reason could be due to the increased SUR2B expression in aorta [48]. Recently, a slow-releasing hydrophilic H<sub>2</sub>S compound GYY4137 has been demonstrated to display vasorelaxing effect in rat endothelium-intact aortic rings and perfused rat renal vasculature through stimulation of vascular K<sub>ATP</sub> channels[49]. Because GYY4137 reduces blood pressure in hypertensive rats without changing heart rate or contracting force *in vitro*, it could be a promising drug for anti-hypertension therapy in future.

## 5.2 Hormones and neurotransmitters

Vascular K<sub>ATP</sub> channels are regulated by many hormones and neurotransmitters (Table 1). Based on vaso-active functions, these endogenous vasoactive substances are classified into two groups: vasoconstrictors and vasodilators. The receptors of these substances are coupled to G<sub>q</sub> and G<sub>s</sub> respectively. G<sub>q</sub> activation stimulates phospholipase C (PLC), which converts membrane phospholipids to diacylglycerol (DAG) and inositol triphosphate (IP<sub>3</sub>). DAG subsequently activates protein kinase C (PKC). G<sub>s</sub> activation stimulates adenylyl cyclase, which catalyzes ATP to produce cAMP. The elevated cAMP in turn binds to regulatory subunits of PKA, leading to PKA dissociation and release of the catalytic subunits. Vascular K<sub>ATP</sub> channels are substrates of both PKC and PKA (see below).

Understanding the molecular mechanism underlying how these vasoactive substances regulate K<sub>ATP</sub> channel is important. As an example, arginine vasopressin (AVP, 0.01–0.04 U/min) was recommended for management of sepsis to avoid using high concentration of catecholamines (e.g. norepinephrine, dopamine) [50] and improve insufficiency of AVP secretion in septic patients[51, 52]. However, a dose higher than 0.03 U/min is not recommend since it may induce coronary vasoconstriction and impair cardiac function[53]. Studies using exogenous expression system show that vascular K<sub>ATP</sub> channel activity is inhibited by AVP [54]. AVP binds to V1a receptor and activates PKC leading to a reduced channel open probability. Therefore, it is likely that AVP elevates blood pressure by inhibiting K<sub>ATP</sub> channels located in peripheral blood vessels, and decreases coronary perfusion through inhibiting coronary arterial K<sub>ATP</sub> channels.

## 5.3 Post-translational regulation

**5.3.1 PKC pathway**—As shown in Table 1, most vasoconstrictors inhibit Kir6.1/SUR2B channel activity through G<sub>q</sub> protein coupled receptor stimulation which results in PKC activation. Patch clamp recording shows that Kir6.1/SUR2B channel rather than Kir6.2/SUR2B channel is sensitive to PKC activation, suggesting Kir6.1 subunit is the target of PKC[64]. Further investigation shows that a motif containing Ser379, Ser385, Ser391 and Ser397 at the distal C-terminus of Kir6.1 is phosphorylated by PKC (Fig. 1) [64]. The 4 serine residues display a repeated pattern (SXRR/KXN) where the underlined S are the phosphorylation sites. The channel inhibition by PKC is slightly reduced when either of the serine residues is mutated to unphosphorylatable alanine. The PKC effect is gradually diminished when more serines are mutated. The PKC inhibition is almost completely eliminated when all the 4 residues are mutated. The additive effects of the repeating PKC sites may allow the channel activity to be elaborately regulated according to the levels of PKC stimulation. PKC activation may also result in caveolin-1 dependent internalization of

vascular  $K_{ATP}$  channel [65], which might occur after the immediate response of channel inhibition and act as a long-term effect of PKC modulation.

**5.3.2 PI3K-Akt pathway**—The inhibitory effect of PKC on vascular  $K_{ATP}$  channel could be reduced by activation of phosphatidylinositol 3-kinases (PI3K)-Akt pathway. Phenylephrine, an  $\alpha$  adrenergic receptor agonist that stimulates PKC, increases Akt phosphorylation in rat endothelium-denuded aorta. A PI3K inhibitor LY294002 enhances levcromakalim-induced vasodilation in aortic rings after pre-exposure to phenylephrine. The levcromakalim-induced  $K_{ATP}$  currents in the presence of phenylephrine are enhanced by an  $\alpha$  adrenergic receptor blocker phentolamine and LY294002 [66]. Therefore, PI3K-Akt pathway may provide a negative feedback to regulate vascular  $K_{ATP}$  channel upon PKC activation. The PI3K-Akt pathway may also be involved in the high glucose-induced vascular  $K_{ATP}$  channel dysfunction. Human endothelium-denuded omental artery treated with *D*-glucose (20 mmol/L) shows increased membrane expression of PI3K p85  $\alpha$  subunit and nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase subunits (p47phox, p22phox, and Rac-1), and elevated Akt phosphorylation as well as intracellular superoxide ( $O_2^-$ ) production. High glucose impairs levcromakalim-induced vasorelaxation and cell membrane hyperpolarization, but can be antagonized by LY294002 or  $O_2^-$  generation inhibitors (tiron and apocynin) [67].

**5.3.3 PKA pathway**—Most vasodilators increase Kir6.1/SUR2B channel activity by stimulating  $G_s$  protein coupled receptors leading to cAMP elevation and PKA activation. Quinn *et al.* have showed that PKA directly phosphorylates Kir6.1/SUR2B channels at 3 residues (Kir6.1 S385, SUR2B T633 and S1465) [68]. Our recent data have showed that 2 different serine residues (Ser1351 and Ser1387) located in the NBD2 of SUR2B are necessary for the channel activation. The Ser1387 is phosphorylated in an *in vitro* phosphorylation assay (Fig. 1) [69]. Further SUR2B modeling study based on the TMD topology of ABC protein SAV1866 suggests that Ser1387 is located on the interface of NBD2 with TMD1 and physically interacted with Tyr506 in TMD1 [16]. A positively charged residue (Arg1462) in NBD2 is close to Ser1387. The three residues produce compact triad upon PKA phosphorylation on Ser1387, leading to reshaping of the NBD2 interface and interdomain movement of NBD2 and TMD1 (Fig. 2). Mutation in any of the three residues diminishes PKA-dependent channel activation. Therefore, Ser1387 phosphorylation increases the NBD-TMD coupling efficiency, and opens the channel.

cAMP also stimulates exchange protein directly activated by cAMP (Epac) which co-localizes with vascular  $K_{ATP}$  channel subunits and inhibit the channel activity via  $Ca^{2+}$ -sensitive protein phosphatase 2B (calcineurin). The concentration of cAMP required to stimulate Epac is higher than PKA [70]. Therefore, cAMP shows two phases of regulation on vascular  $K_{ATP}$  channels: cAMP at low concentration activates the  $K_{ATP}$  channel via PKA, while a high concentration of cAMP inhibits channel activity through Epac.

Another interesting aspect is that the PKA-dependent Kir6.1/SUR2B channel activation can be antagonized by calcineurin [71]. The mechanisms may be due to: (1) calcineurin reduces PKA activity leading to an indirect channel inhibition; (2) calcineurin directly dephosphorylates the channel. However, it is unknown if calcineurin can modulate the PKA

phosphorylation sites at SUR2B. Nevertheless, calcineurin seems to balance the effects of cAMP/PKA on vascular  $K_{ATP}$  channels.

#### 5.3.4 Reactive oxygen species (ROS) and S-glutathionylation—An

overproduction of ROS is one of the characteristics of oxidative stress which contributes to the development of many types of diseases, such as diabetes, atherosclerosis and sepsis [72]. The effect of ROS on  $K_{ATP}$  channel has been noticed recently. Cerebral arterioles treated with  $O_2^-$  show a less vasorelaxation response to cromakalim [73]. A pre-exposure of isolated mesenteric arterial rings to  $H_2O_2$  also attenuates the  $K_{ATP}$  channel-mediated vasorelaxation [74]. In a diabetic rat model, pinacidil-induced vasodilation in cerebral arterioles is impaired, but is completely restored by treatments with superoxide dismutase (SOD) and catalase [75]. Similar impaired vascular responses to  $K_{ATP}$  channel openers are also observed in diabetic patients [76]. Therefore, the ROS overproduction in oxidative stress disrupts vascular  $K_{ATP}$  activity.

Recent studies have showed that  $H_2O_2$  induces a glutathione (GSH) dependent Kir6.1/SUR2B channel inhibition, which can be mimicked by oxidized glutathione (GSSG) or thiol-modulating reagents [74]. The oxidant-mediated channel suppression is rescued by the reducing agent dithiothreitol (DTT) and the specific deglutathionylation agent glutaredoxin-1 (Grx1), suggesting S-glutathionylation is a mechanism underlying the channel modulation in oxidative stress. Moreover, it has been identified that Cys43 in N-terminus, Cys120 and Cys176 in the transmembrane domain of Kir6.1, accounts for the oxidant sensitivity. Among the 3 residue, Cys176 makes a major contribution (Fig. 1). Using structural modeling, how the addition of GSH to the channel protein can affect the gating of  $K_{ATP}$  channel has been studied. Simulation modeling suggests that after binding residue Cys176, the GSH moiety occupies a space between the slide helix and two transmembrane helices. Since the gating of  $K_{ATP}$  channel requires the movement of inner transmembrane helix, the addition of GSH in this critical location limits the conformational changes of the inner transmembrane helix, thus impairs channel gating and retains the channel in its closed state (Fig. 3) [77].

#### 5.4 Transcriptional regulation

The expression of  $K_{ATP}$  channel subunits could be altered in some medical conditions and diseases. For instance, a declined SUR2B mRNA instead of Kir6.1 and Kir6.2 is observed in SMCs dissociated from diabetic rat aorta [78]. The Kir6.1 and SUR2B expression (mRNA and protein) in aortic SMCs, as well as the isolated  $K_{ATP}$  current density, are also reduced in obese rats [79]. Flow stress elevates the expression of Kir6.2 (both mRNA and protein) in rat pulmonary microvascular endothelial cells [80].

The  $K_{ATP}$  channel activity may also be subject to chronic hypoxic regulation. Glibenclamide reduces vasorelaxation of pial arterioles during hypoxia *in vivo* [81]. The diazoxide-induced vasodilation in near-term pregnant uterine arteries is compromised after long-term exposure to hypoxia at high altitude [82]. The molecular mechanism underlying the causal relationship between hypoxia and vascular  $K_{ATP}$  channel is not well examined. However, the mRNA and protein expression of Kir6.1 and Kir6.2 are up-regulated under venous hypoxemia in right



atrium of patients with tetralogy of Fallot or ventricular septal defects. The study shows that Kir6.1 mRNA transcription is Forkhead box (FOX) O1 dependent, whereas FOXO1 transcription is hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) dependent. Moreover, the study on cultured rat atrial myocytes also confirmed the causal relationships among hypoxia, HIF-1 $\alpha$ , FOXO1, and Kir6.1 [83].

K<sub>ATP</sub> channel exhibits a high channel activity in sepsis. Glibenclamide has been tested in several septic animal models and shows to raise blood pressure through increasing systemic vascular resistance<sup>[84, 85]</sup>. PNU-37883A, an K<sub>ATP</sub> channel inhibitor targeting the pore region of Kir6 subunit, displays more potent inhibitory effect on Kir6.1/SUR2B than Kir6.2/SUR2B<sup>[86]</sup>, and provides better outcomes to reverse LPS-induced vascular hyporeactivity to circulating epinephrine<sup>[87]</sup>. The high channel activity is mainly due to upregulation of K<sub>ATP</sub> channel expression. Both mRNA and protein levels for Kir6.1 are increased in the diaphragm of rats treated with LPS, with the mRNA level increased by 4-fold in 48 h, whereas protein levels augmented 9-fold after 24 h<sup>[88]</sup>. Moreover, Kir6.1 expression in colonic smooth muscle is enhanced by 22-fold, the mRNA level for SUR2B is decreased by 3-fold in experimental colitis<sup>[89]</sup>. We reveal that an overnight LPS treatment hyperpolarizes aortic SMCs. Whole cell patch clamping shows that K<sub>ATP</sub> current density is elevated in aortic SMCs exposed to LPS, but not changed in HEK293 cells heterologously expressing Kir6.1/SUR2B. The increased protein surface expression is due to nuclear factor (NF)- $\kappa$ B-dependent Kir6.1 and SUR2B mRNA expression<sup>[90]</sup>. Such an upregulation increases K<sub>ATP</sub> channel activity, and may lead to excessive vasodilation during sepsis. A recent study using a rat septic shock model induced by peritonitis shows that septic vascular hyporeactivity is improved by PNU-37883A but not high-conductance Ca<sup>2+</sup>-activated K<sup>+</sup> (BK<sub>Ca</sub>) channel blocker IbTX. In consistent, sepsis increases mRNA and protein expression of Kir6.1 and SUR2B subunits, but does not change expression of BK<sub>Ca</sub> channels. The elevated aortic NO release, NF- $\kappa$ B activation, and K<sub>ATP</sub> channel upregulation are inducible NOS dependent<sup>[91]</sup>. These observations again suggest that a transcriptional mechanism underlying the K<sub>ATP</sub> channel regulation during sepsis, and indicate that selectively inhibiting vascular K<sub>ATP</sub> channel could offer promising therapeutic approaches to manage septic shock.

## 5.5 Non-sulfonylurea anti-diabetic agents

Some anti-diabetic agents that are not categorized in sulfonylurea family have been demonstrated to affect vascular K<sub>ATP</sub> channel activity recently. Rosiglitazone (RSG) reduces blood glucose level by increasing insulin sensitivity and glucose uptake in skeletal muscle and adipose tissues. Our recent study have showed that RSG is not only able to inhibit the Kir6.1/SUR2B channels in a membrane-delimited manner, but also attenuate the adrenergic mediated coronary vasodilation<sup>[92]</sup>. The vasoactive effect is Kir6.1 dependent, because the isolated hearts from Kir6.1 knockout mice show less response to RSG. Phenformin is a biguanide that used to treat type II diabetes mellitus. It is more selective to block Kir6.1/SUR2B channel, with a 90% reduction of open probability in inside out patch, and also inhibit the K<sub>ATP</sub> current in native vascular SMCs<sup>[93]</sup>. These results suggest that RSG and phenformin not only reduce blood glucose, but also act as vascular K<sub>ATP</sub> channel inhibitors, and may potentially reduce coronary response to circulating vasodilators and metabolic stress.

## 6 Summary

Ample evidence from experimental animal models and the identification of  $K_{ATP}$  channel mutations in patients indicate that  $K_{ATP}$  channel plays a critical role in vascular tone regulation, and likely contributes to the pathogenesis of many cardiovascular diseases. It remains challenging to design therapeutic modalities based on an intervention to the  $K_{ATP}$  channel. For an example, LPS-induced vascular  $K_{ATP}$  channel upregulation may be a myocardial protective mechanism because it increases coronary blood flow and reduces myocardial depression during sepsis. However, an excessively up-regulated vascular  $K_{ATP}$  channel will cause severe peripheral vasodilation leading to lethal hypotension and organ failure. The two contradictory effects of the  $K_{ATP}$  channel on coronary and systemic circulations hinder the administration of  $K_{ATP}$  channel blockers and openers in sepsis in which both the reasonable controls of systemic vascular contractility and the maintenance of coronary circulation are necessary. All of these depend on the understanding of the molecular mechanisms underlying the vascular  $K_{ATP}$  channel regulation.

## Acknowledgments

This work was supported by National Heart, Lung, and Blood Institute Grant (No. HL-067890), National Institutes of Health Grant (No. HD060959), and the American Heart Association (No. 09GRNT2010037).

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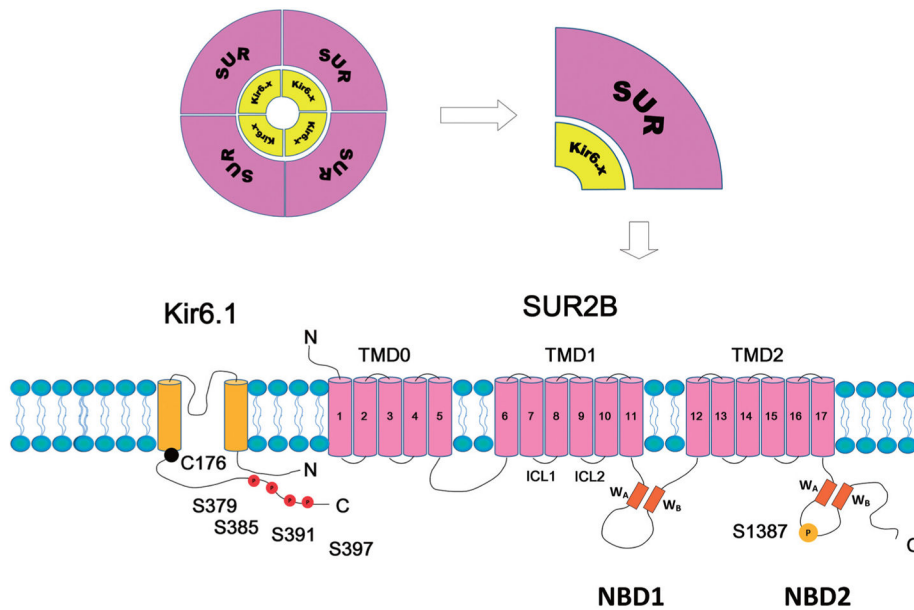
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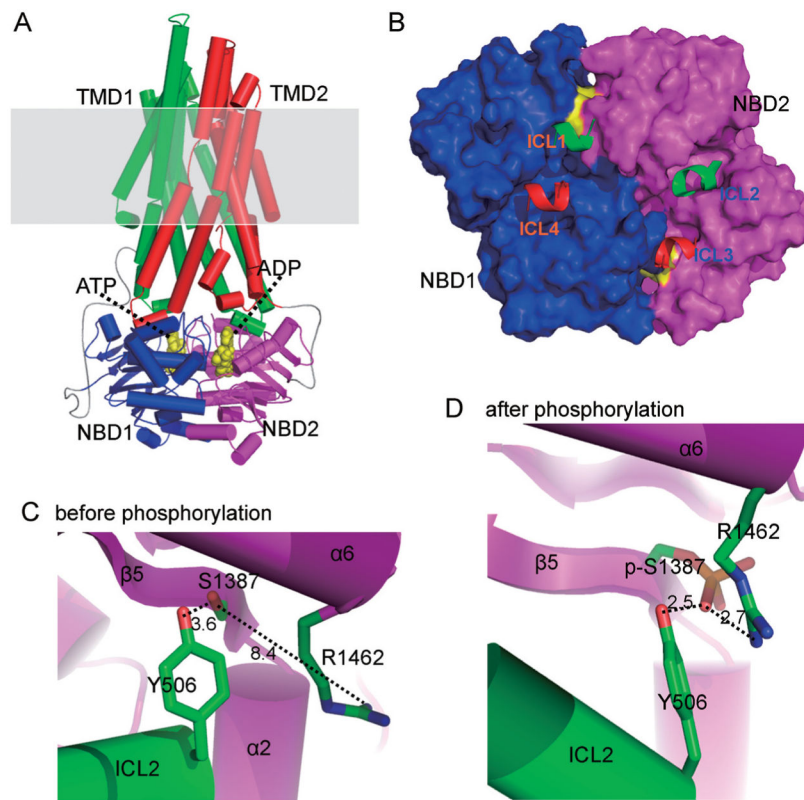
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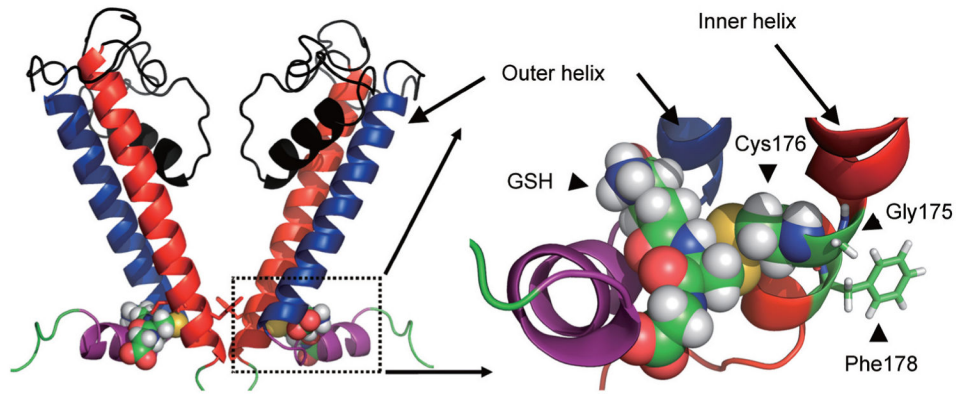


**Fig. 1.** Molecular structure of the vascular  $K_{ATP}$  channel.  $K_{ATP}$  channels are octameric complexes formed by 4 Kir6 subunits (Kir6.x) and 4 accessory sulfonylurea receptor (SUR) subunits. The Kir6.x subunit has 2 transmembrane helices. SUR subunit has 3 transmembrane domains (TMD0, 1 and 2). There are two intracellular loops linking the adjacent TMDs. Each intracellular loop contains a nucleotide binding domain (NBD1 and NBD2, respectively). A Walker A motif ( $W_A$ ), a Walker B motif ( $W_B$ ), and a linker region are located within the NBDs. The Ser379, Ser385, Ser391, and Ser397 at the distal C-terminus of Kir6.1 are PKC phosphorylation residues. The Ser1387 is PKA phosphorylation residue. The Cys176 in the transmembrane domain of Kir6.1 is the major residue accounting for the channel's oxidant sensitivity.



**Fig. 2.**

Model of SUR2B core domains. *A*: SUR2B core domains (TMD1, 2 and NBD1, 2) are modeled using SAV1866 as a template. Shaded region is plasmic membrane. *B*: The interface between TMDs and NBDs are highlighted. Intracellular loop-1 (ICL1) of TMD1 physically interacts with NBD1 and NBD2. ICL2 of TMD1 only interacts with NBD2. Thus TMD1 mainly interact with NBD2. Similarly, TMD2 mainly interacts with NBD1. *C* and *D*: The three critical residues involved in PKA phosphorylation are highlighted. Note the side chain of Arg1462 is far from phosphorylation residue Ser1387 before phosphorylation. It is attracted by phosphorylated Ser1387 (p-Ser1387) after PKA phosphorylation and forms a tight triad with p-Ser1387 and Tyr506. The figure is modified from *Journal of Biological Chemistry* with permission <sup>[16]</sup>.



**Fig. 3.** Structural modeling of Kir6.1 protein with the incorporation of GSH. The overall structural model of two opposing Kir6.1 monomers (out of four for clarity) was displayed. Boxed area was enlarged and showed the GSH associated area. The GSH moiety occupies a space between the inner and outer helix. The addition of GSH therefore impairs the movement of inner helix, which is necessary for the channel opening. The figure is modified from *Journal of Biological Chemistry* with permission [77].

**Table 1**Summary of vasoactive substances targeting vascular  $K_{ATP}$  channels

	Vasoactive substances	Receptor	Distributions	References
Vasoconstrictors	Noradrenaline	$\alpha_2$	Rat tail artery	[55]
	Endothelin-1	N/A	Rabbit coronary and pulmonary arteries	[56]
	Angiotension II	N/A	Rat mesenteric artery	[57]
	AVP	$V_{1a}$	Rat mesenteric artery	[54]
	Neuropeptide Y	NPY1	Rabbit mesenteric artery, dog coronary artery	[58, 59]
	Serotonin	5-HT <sub>2</sub>	Rabbit mesenteric artery	[58]
	Histamine	H <sub>1</sub>	Rabbit mesenteric artery	[58]
Vasodilators	Adenosine	A <sub>2</sub>	Rat mesenteric artery, guinea pig coronary artery	[60, 61]
	VIP	VPAC1	Rat mesenteric artery	[62]
	Glucagon-like peptide-1	GLP-1	Rat aorta	[63]
	CGRP	N/A	Rabbit mesenteric artery, pig coronary artery	[36, 37]

N/A, not reported.