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Invited Review

K_{ATP} channel action in vascular tone regulation: from genetics to diseases

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Abstract: ATP-sensitive potassium (K_{ATP}) channels are widely distributed in vasculatures, and play an important role in the vascular tone regulation. The K_{ATP} channels consist of 4 pore-forming inward rectifier K^+ channel (Kir) subunits and 4 regulatory sulfonylurea receptors (SUR). The major vascular isoform of K_{ATP} 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 α-adrenoceptor agonists stimulate protein kinase C (PKC) and inhibit the K_{ATP} channels, while vasodilators such as β-adrenoceptor agonists and vasoactive intestinal polypeptide increase K_{ATP} 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-κB 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_{ATP} channel regulation under 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.

Key words: ATP-sensitive potassium channel; Kir6.1; SUR2B; protein phosphorylation; *S*-glutathionylation; nuclear factor-κB; sepsis; sudden infant death syndrome; J-wave syndrome

ATP敏感钾通道调节血管张力的分子机制

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摘 要：ATP敏感钾通道(ATP-sensitive potassium channel, K_{ATP}通道)广泛分布在血管系统，并在血管张力调节中发挥重要作

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用。 K_{ATP}通道由4个孔道形成的内向整流钾离子通道(inward rectifier K^+ channels, Kir)亚基和4个磺脲受体调节亚基(sulfonylurea receptor, SUR)组成。尽管其它一些亚基在血管中也存在，Kir6.1/SUR2B是主要的血管亚型K_{ATP}通道。K_{ATP}通道转基因小鼠的研究以及人群中K_{ATP}通道基因突变的发现，都强烈支持K_{ATP}通道对于心血管系统的动态平衡调控是不可缺少的。大量的血管活性物质通过调节K_{ATP}通道活性来改变血管平滑肌细胞的膜电位，从而调节血管张力。多数内源性血管收缩物质，例如血管加压素，激活蛋白激酶C (protein kinase C, PKC)，磷酸化K_{ATP}通道并抑制其活性；而血管扩张物质，如血管活性肠肽，通过增加cAMP的形成和提高蛋白激酶A (protein kinase A, PKA)的活性来增加K_{ATP}通道的活性。PKC作用于Kir6.1亚基C-末端，磷酸化4个保守的丝氨酸，而PKA磷酸化SUR2B亚基第2核苷酸结合域的Ser1387位点。血管K_{ATP}通道也受活性氧的调节，其中Kir6.1的Cys176是一个重要的过氧化物调节位点。此外，K_{ATP}通道功能可被一些慢性的病理生理条件上调，如感染性休克。核因子-κB依赖的基因转录是脂多糖诱导的血管K_{ATP}通道激活的一个机制。本综述将概括性描述血管K_{ATP}通道在生理和病理情况下受到的调节，以期阐明血管K_{ATP}通道在治疗和预防心血管疾病方面可能是一个有用的靶点。

关键词：ATP敏感钾通道；内向整流钾离子通道亚基6.1；磺脲受体调节亚基2B；蛋白磷酸化；核因子-κB；脓毒血症；婴儿猝死综合征；J波综合征
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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 myoctes [2] and pancreatic β 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 helixes (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 β 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].

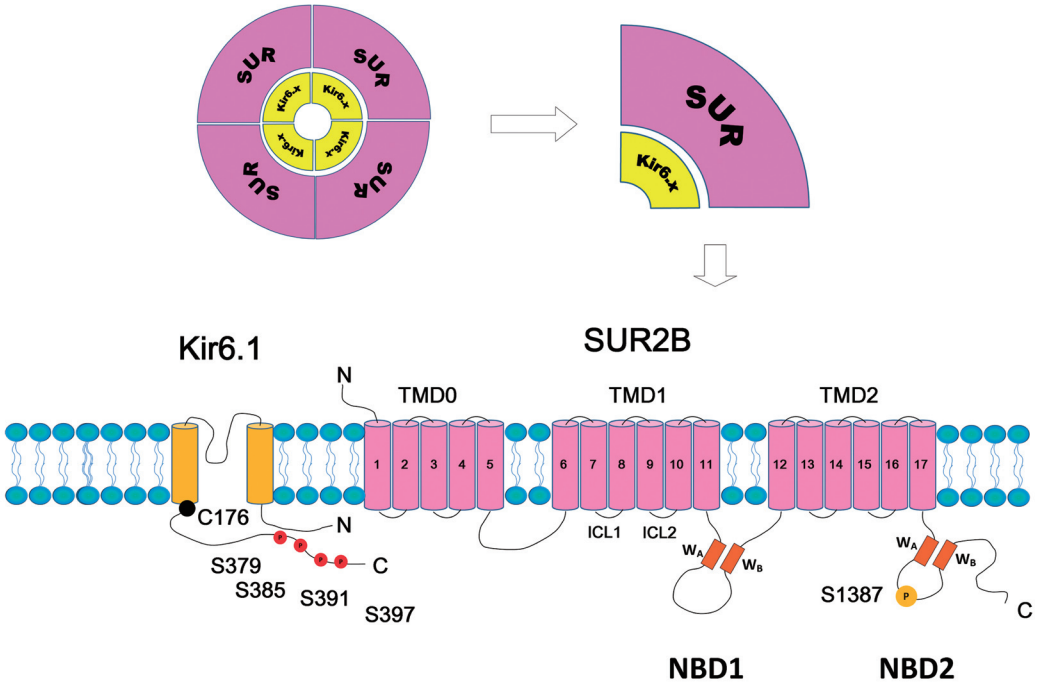

Fig. 1. Molecular structure of the vascular K_{ATP} channel. K_{ATP} channels are octameric complexes formed by 4 Kir6 subunits (Kir6.x) and 4 accessory sulfonylurea receptor (SUR) subunits. The Kir6.x subunit has 2 transmembrane helixes. SUR subunit has 3 transmembrane domains (TMD0, 1 and 2). There 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.

The main body of SUR2B (TMD1, 2 and NBD1, 2) shares many properties with other transmembrane ABCCs. 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[10].

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[19]. 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 [20]. 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) [19]. Similar effects were observed by knockdown of both *Ir* and *Ir2* genes [23]. 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 [23].

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[24]. 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[25]. In comparison, intracellular ADP concentration ranges between 0.1 and 3 mmol/L [25], and exhibits stimulatory effect on K_{ATP} channel. According to this characteristic, vascular K_{ATP} channel was once termed as K_{NDP} channel[5, 26].

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[27, 28]. Hypercapnic acidosis induces vasodilation through activation of K_{ATP} channels in VSMs,

with maximal effect at pH 6.5 to 6.8 [29]. Blockade of K_{ATP} channels attenuates the vasodilation, which is observed in cerebral arterioles, basil artery, coronary artery, mesenteric artery or internal mammary artery. The modulations of K_{ATP} channel activity by H^+, ATP and ADP are mediated via direct ligand binding to Kir6.x or SUR, leading to alternation in the channel gating [30–32].

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_{ATP} channels[33]. NO released from skeletal muscle vasculatures during excise may activate vascular K_{ATP} channels, and antagonizes sympathetic vasoconstriction, providing a delicate mechanism to regulate blood flow in exercising skeletal muscles[34]. Lactate, an important metabolic product in retina, relaxes retinal arterioles through activation of nitric oxide synthase (NOS) and guanylyl cyclase, and opening of K_{ATP} channel [35]. However, it is also reported that NO donor sodium nitroprusside fails to activate K_{ATP} currents isolated from rabbit mesenteric arterial SMCs and pig coronary arterial SMCs [36, 37]. Therefore, the exact role of NO in regulating vascular K_{ATP} channels is still debatable.

5.1.4 Eicosanoids

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

5.1.5 Hydrogen sulfide (H_2S)

H_2S is a product from *L*-cysteine metabolism catalyzed by cystathionine-γ-lyase and cystathionine-β-synthase in mammalian tissues. Endogenous H_2S has been detected in various vascular tissues (e.g. aorta, tail, and mesenteric arteries) [43]. H_2S in physiological concentrations (nearly 45 μmol/L) induces vasodilation in rat aorta and transient reduction of blood pressure through activation of K_{ATP} channels [44, 45]. Pinacidil- and H_2S-induced vasorelaxation are compromised in cerebral arterioles of SUR2-null mice[46]. Patch clamping studies demonstrate that exogenous H_2S activates K_{ATP} channels and hyperpolarizes cell membrane in SMC isolated from rat mesenteric artery [47] and piglet cerebral arterioles [46]. In addition, aortic rings seem to be more sensitive to H_2S than pulmonary arterial rings. The reason could be due to the increased SUR2B expression in aorta [48]. Recently, a slow-releasing hydrophilic H_2S 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_{ATP} 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_{ATP} channels are regulated by many hormones and neurotransmitters (Table 1). Based on vasoactive functions, these endogenous vasoactive sub-

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

	Vasoactive substances	Receptor	Distributions	References
Vasoconstrictors	Noradrenaline	α_2	Rat tail artery	[55]
	Endothelin-1	N/A	Rabbit coronary and pulmonary arteries	[56]
	Anginotension 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_2	Rabbit mesenteric artery	[58]
	Histamine	H_1	Rabbit mesenteric artery	[58]
Vasodilators	Adenosine	A_2	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.

stances are classified into two groups: vasoconstrictors and vasodilators. The receptors of these substances are coupled to G_q and G_s respectively. G_q activation stimulates phospholipase C (PLC), which converts membrane phospholipids to diacylglycerol (DAG) and inositol triphosphate (IP_3). DAG subsequentially activates protein kinase C (PKC). G_s 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_{ATP} channels are substrates of both PKC and PKA (see below).

Understanding the molecular mechanism underlying how these vasoactive substances regulate K_{ATP} channel is important. As an example, arginine vasopression (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 recommended since it may induce coronary vasoconstriction and impair cardiac function[53]. Studies using exogenous expression system show that vascular K_{ATP} 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_{ATP} channels located in peripheral blood vessels, and decreases coronary perfusion through inhibiting coronary arterial K_{ATP} channels.

5.3 Post-translational regulation

5.3.1 PKC pathway

As shown in Table 1, most vasoconstrictors inhibit Kir6.1/SUR2B channel activity though G_q 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 (S*X*RR/K*X*N) 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 unphosphorylable 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 α 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 α 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 α 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 inhibits 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-

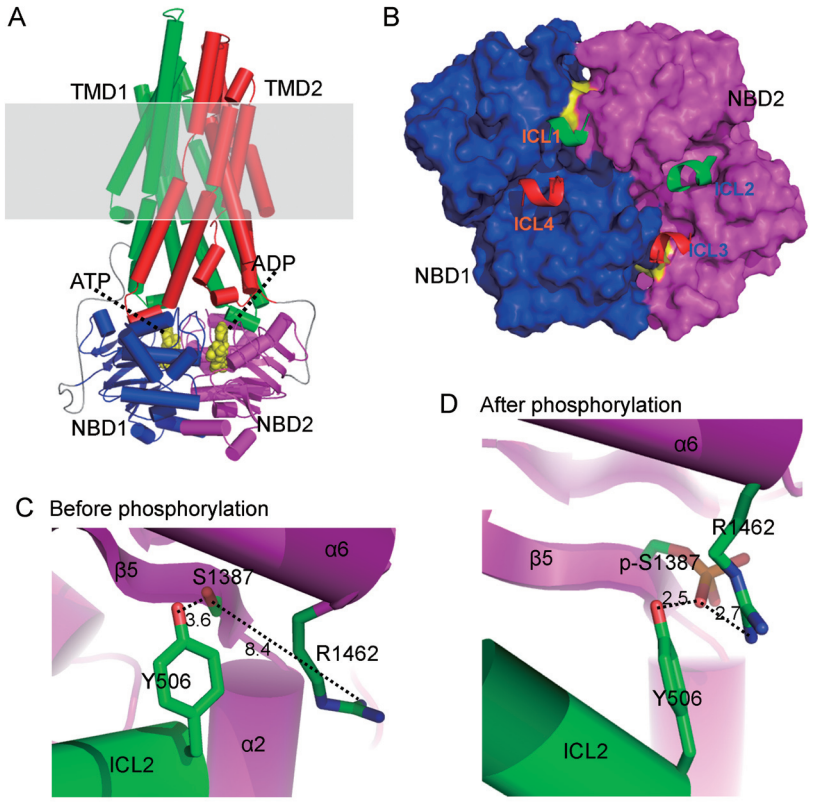

Fig. 2. Model of SUR2B core domains. *A*: SUR2B core domains (TMD1, 2 and NBD1, 2) are modeled using SAV1866 as a template. Shaded region is plasmic membrane. *B*: The interface between TMDs and NBDs are highlighted. Intracellular loop-1 (ICL1) of TMD1 physically interacts with NBD1 and NBD2. ICL2 of TMD1 only interacts with NBD2. Thus TMD1 mainly interacts 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 phosphorlation 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 [16].

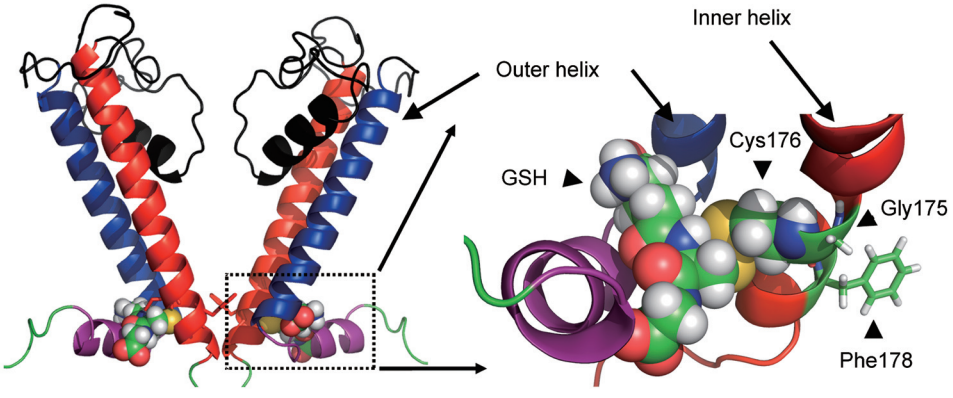

Fig. 3. Structural modeling of Kir6.1 protein with the incorporation of GSH. The overall structural model of two opposing Kir6.1 monomers (out of four for clarity) was displayed. Boxed area was enlarged and showed the GSH associated area. The GSH moiety occupies a space between the inner and outer helix. 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].

inducible factor-1α (HIF-1α) dependent. Moreover, the study on cultured rat atrial myocytes also confirmed the causal relationships among hypoxia, HIF-1α, FOXO1, and Kir6.1 [83].

K_{ATP} 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[84, 85]. PNU-37883A , an K_{ATP} channel inhibitor targeting the pore region of Kir6 subunit, displays more potent inhibitory effect on Kir6.1/SUR2B than Kir6.2/SUR2B[86], and provides better outcomes to reverse LPS-induced vascular hyporeactivity to circulating epinephrine [87]. The high channel activity is mainly due to upregulation of K_{ATP} 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[88]. Moreover, Kir6.1 expression in colonic smooth muscle is enhanced by 22-fold, and the mRNA level for SUR2B is decreased by 3-fold in experimental colitis[89]. We reveal that an overnight LPS treatment hyperpolarizes aortic SMCs. Whole cell patch clamping shows that K_{ATP} 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)-κB-dependent Kir6.1 and SUR2B mRNA expression[90]. Such an upregulation increases K_{ATP} 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^{2+}-activated K^+ (BK_{Ca}) channel blocker IbTX. In consistent, sepsis increases mRNA and protein expression of Kir6.1 and SUR2B subunits, but does not change expression of BK_{Ca} channels. The elevated aortic NO release, NF-κB activation, and K_{ATP} channel upregulation are inducible NOS dependent[91]. These observations again suggest that a transcriptional mechanism underlying the K_{ATP} channel regulation during sepsis, and indicate that selectively inhibiting vascular K_{ATP} 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_{ATP} 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[92]. 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_{ATP} current in native vascular SMCs[93]. These results suggest that RSG and phenformin not only reduce blood glucose, but also act as vascular K_{ATP} 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 upregulated 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.

REFERENCES

1 Noma A. ATP-regulated K^+ channels in cardiac muscle. Nature 1983; 305(5930): 147–148.

2 Spruce AE, Standen NB, Stanfield PR. Voltage-dependent ATP-sensitive potassium channels of skeletal muscle membrane. Nature 1985; 316(6030): 736–738.

3 Trube G, Rorsman P, Ohno-Shosaku T. Opposite effects of tolbutamide and diazoxide on the ATP-dependent K^+ channel in mouse pancreatic beta-cells. Pflugers Arch 1986; 407(5): 493–499.

4 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(4914): 177–180.

5 Beech DJ, Zhang H, Nakao K, Bolton TB. K channel activation by nucleotide diphosphates and its inhibition by glibenclamide in vascular smooth muscle cells. Br J Pharmacol 1993; 110(2): 573–582.

6 Nelson MT, Huang Y, Brayden JE, Hescheler J, Standen NB. Arterial dilations in response to calcitonin gene-related peptide involve activation of K^+ channels. Nature 1990; 344(6268): 770–773.

7 Winquist RJ, Heaney LA, Wallace AA, Baskin EP, Stein RB, Garcia ML, Kaczorowski GJ. Glyburide blocks the relaxation response to BRL 34915 (cromakalim), minoxidil sulfate and diazoxide in vascular smooth muscle. J Pharmacol Exp Ther 1989; 248(1): 149–156.

8 Haissaguerre M, Chatel S, Sacher F, Weerasooriya R, Probst V, Loussouarn G, Horlitz M, Liersch R, Schulze-Bahr E, Wilde A, Kaab S, Koster J, Rudy Y, Le Marec H, Schott JJ. Ventricular fibrillation with prominent early repolarization associated with a rare variant of KCNJ8/K_{ATP} channel. J Cardiovasc Electrophysiol 2009; 20(1): 93–98.

9 Medeiros-Domingo A, Tan BH, Crotti L, Tester DJ, Eckhardt L, Cuoretti A, Kroboth SL, Song C, Zhou Q, Kopp D, Schwartz PJ, Makielski JC, Ackerman MJ. Gain-of-function mutation S422L in the KCNJ8-encoded cardiac K_{ATP} channel Kir6.1 as a pathogenic substrate for J-wave syndromes. Heart Rhythm 2010; 7(10): 1466–1471.

10 Tester DJ, Tan BH, Medeiros-Domingo A, Song C, Makielski JC, Ackerman MJ. Loss-of-function mutations in the KCNJ8-encoded Kir6.1 K_{ATP} channel and sudden infant death syndrome. Circ Cardiovasc Genet 2011; 4(5): 510–515

11 Campbell JD, Sansom MS, Ashcroft FM. Potassium channel regulation. EMBO Rep 2003; 4(11): 1038–1042.

12 Yamada M, Isomoto S, Matsumoto S, Kondo C, Shindo T, Horio Y, Kurachi Y. Sulphonylurea receptor 2B and Kir6.1 form a sulphonylurea-sensitive but ATP-insensitive K^+ channel. J Physiol 1997; 499 (Pt 3): 715–720.

13 Isomoto S, Kondo C, Yamada M, Matsumoto S, Higashiguchi O, Horio Y, Matsuzawa Y, Kurachi Y. A novel sulfonylurea receptor forms with BIR (Kir6.2) a smooth muscle type ATP-sensitive K^+ channel. J Biol Chem 1996; 271(40): 24321–24324.

14 Ammala C, Moorhouse A, Gribble F, Ashfield R, Proks P, Smith PA, Sakura H, Coles B, Ashcroft SJ, Ashcroft FM. Promiscuous coupling between the sulphonylurea receptor and inwardly rectifying potassium channels. Nature 1996; 379(6565): 545–548.

15 Chutkow WA, Simon MC, Le Beau MM, Burant CF. Cloning, tissue expression, and chromosomal localization of SUR2, the putative drug-binding subunit of cardiac, skeletal muscle, and vascular K_{ATP} channels. Diabetes 1996; 45(10): 1439–1445.

16 Shi Y, Chen X, Wu Z, Shi W, Yang Y, Cui N, Jiang C, Harrison RW. cAMP-dependent protein kinase phosphorylation produces interdomain movement in SUR2B leading to activation of the vascular K_{ATP} channel. J Biol Chem 2008; 283(12): 7523–7530.

17 Chutkow WA, Pu J, Wheeler MT, Wada T, Makielski JC, Burant CF, McNally EM. Episodic coronary artery vasospasm and hypertension develop in the absence of Sur2 K_{ATP} channels. J Clin Invest 2002; 110(2): 203–208.

18 Miki T, Suzuki M, Shibasaki T, Uemura H, Sato T, Yamaguchi K, Koseki H, Iwanaga T, Nakaya H, Seino S. Mouse model of Prinzmetal angina by disruption of the inward rectifier Kir6.1. Nat Med 2002; 8(5): 466–472.

19 Croker B, Crozat K, Berger M, Xia Y, Sovath S, Schaffer L, Eleftherianos I, Imler JL, Beutler B. ATP-sensitive potassium channels mediate survival during infection in mammals and insects. Nat Genet 2007; 39(12): 1453–1460.

20 Kane GC, Lam CF, O'Cochlain F, Hodgson DM, Reyes S, Liu XK, Miki T, Seino S, Katusic ZS, Terzic A. Gene knockout of the KCNJ8-encoded Kir6.1 K_{ATP} channel imparts fatal susceptibility to endotoxemia. FASEB J 2006; 20(13): 2271–2280.

21 Kakkar R, Ye B, Stoller DA, Smelley M, Shi NQ, Galles K, Hadhazy M, Makielski JC, McNally EM. Spontaneous coronary vasospasm in K_{ATP} mutant mice arises from a smooth muscle-extrinsic process. Circ Res 2006; 98(5): 682–689.

22 Malester B, Tong X, Ghiu I, Kontogeorgis A, Gutstein DE, Xu J, Hendricks-Munoz KD, Coetzee WA. Transgenic expression of a dominant negative K_{ATP} channel subunit in the mouse endothelium: effects on coronary flow and endothelin-1 secretion. FASEB J 2007; 21(9): 2162–2172.

23 Eleftherianos I, Won S, Chtarbanova S, Squiban B, Ocorr K, Bodmer R, Beutler B, Hoffmann JA, Imler JL. ATP-sensitive potassium channel (K_{ATP})-dependent regulation of cardiotropic viral infections. Proc Natl Acad Sci U S A 2011; 108(29): 12024–12029.

24 Quayle JM, Nelson MT, Standen NB. ATP-sensitive and inwardly rectifying potassium channels in smooth muscle. Physiol Rev 1997; 77(4): 1165–1232.

25 Randak CO, Welsh MJ. ADP inhibits function of the ABC transporter cystic fibrosis transmembrane conductance regulator via its adenylate kinase activity. Proc Natl Acad Sci U S A 2005; 102(6): 2216–2220.

26 Cole WC, Malcolm T, Walsh MP, Light PE. Inhibition by

protein kinase C of the K_{NDP} subtype of vascular smooth muscle ATP-sensitive potassium channel. Circ Res 2000; 87(2): 112–117.

27 Kontos HA, Raper AJ, Patterson JL. Analysis of vasoactivity of local pH, PCO_2 and bicarbonate on pial vessels. Stroke 1977; 8(3): 358–360.

28 Tian R, Vogel P, Lassen NA, Mulvany MJ, Andreasen F, Aalkjaer C. Role of extracellular and intracellular acidosis for hypercapnia-induced inhibition of tension of isolated rat cerebral arteries. Circ Res 1995; 76(2): 269–275.

29 Wang X, Wu J, Li L, Chen F, Wang R, Jiang C. Hypercapnic acidosis activates K_{ATP} channels in vascular smooth muscles. Circ Res 2003; 92(11): 1225–1232.

30 Matsuo M, Tanabe K, Kioka N, Amachi T, Ueda K. Different binding properties and affinities for ATP and ADP among sulfonylurea receptor subtypes, SUR1, SUR2A, and SUR2B. J Biol Chem 2000; 275(37): 28757–28763.

31 Dabrowski M, Tarasov A, Ashcroft FM. Mapping the architecture of the ATP-binding site of the K_{ATP} channel subunit Kir6.2. J Physiol 2004; 557(Pt 2): 347–354.

32 Xu H, Cui N, Yang Z, Wu J, Giwa LR, Abdulkadir L, Sharma P, Jiang C. Direct activation of cloned K_{ATP} channels by intracellular acidosis. J Biol Chem 2001; 276(16): 12898–12902.

33 Murphy ME, Brayden JE. Nitric oxide hyperpolarizes rabbit mesenteric arteries via ATP-sensitive potassium channels. J Physiol 1995; 486 (Pt 1): 47–58.

34 Thomas GD, Victor RG. Nitric oxide mediates contraction-induced attenuation of sympathetic vasoconstriction in rat skeletal muscle. J Physiol 1998; 506 (Pt3): 817–826.

35 Hein TW, Xu W, Kuo L. Dilation of retinal arterioles in response to lactate: role of nitric oxide, guanylyl cyclase, and ATP-sensitive potassium channels. Invest Ophthalmol Vis Sci 2006; 47(2): 693–699.

36 Quayle JM, Bonev AD, Brayden JE, Nelson MT. Calcitonin gene-related peptide activated ATP-sensitive K^+ currents in rabbit arterial smooth muscle via protein kinase A. J Physiol 1994; 475(1): 9–13.

37 Wellman GC, Quayle JM, Standen NB. ATP-sensitive K^+ channel activation by calcitonin gene-related peptide and protein kinase A in pig coronary arterial smooth muscle. J Physiol 1998; 507(Pt1): 117–129.

38 Rosolowsky M, Campbell WB. Synthesis of hydroxyeicosatetraenoic (HETEs) and epoxyeicosatrienoic acids (EETs) by cultured bovine coronary artery endothelial cells. Biochim Biophys Acta 1996; 1299(2): 267–277.

39 Archer SL, Gragasin FS, Wu X, Wang S, McMurtry S, Kim DH, Platonov M, Koshal A, Hashimoto K, Campbell WB, Falck JR, Michelakis ED. Endothelium-derived hyperpolarizing factor in human internal mammary artery is 11,12-epoxyeicosatrienoic acid and causes relaxation by activating smooth muscle BK_{Ca} channels. Circulation 2003; 107(5): 769–776.

40 Campbell WB, Falck JR. Arachidonic acid metabolites as endothelium-derived hyperpolarizing factors. Hypertension 2007; 49(3): 590–596.

41 Ye D, Zhou W, Lu T, Jagadeesh SG, Falck JR, Lee HC. Mechanism of rat mesenteric arterial K_{ATP} channel activation by 14, 15-epoxyeicosatrienoic acid. Am J Physiol Heart Circ Physiol 2006; 290(4): H1326–1336.

42 Ye D, Zhou W, Lee HC. Activation of rat mesenteric arterial K_{ATP} channels by 11, 12-epoxyeicosatrienoic acid. Am J Physiol Heart Circ Physiol 2005; 288(1): H358–H364.

43 Cheng Y, Ndisang JF, Tang G, Cao K, Wang R. Hydrogen sulfide-induced relaxation of resistance mesenteric artery beds of rats. Am J Physiol Heart Circ Physiol 2004; 287(5): H2316–H2323.

44 Zhao W, Wang R. H_2S-induced vasorelaxation and underlying cellular and molecular mechanisms. Am J Physiol Heart Circ Physiol 2002; 283(2): H474–H480.

45 Zhao W, Zhang J, Lu Y, Wang R. The vasorelaxant effect of H_2S as a novel endogenous gaseous K_{ATP} channel opener. EMBO J 2001; 20(21): 6008–6016.

46 Liang GH, Adebiyi A, Leo MD, McNally EM, Leffler CW, Jaggar JH. Hydrogen sulfide dilates cerebral arterioles by activating smooth muscle cell plasma membrane K_{ATP} channels. Am J Physiol Heart Circ Physiol 2011; 300(6): H2088–H2095.

47 Tang G, Wu L, Liang W, Wang R. Direct stimulation of K_{ATP} channels by exogenous and endogenous hydrogen sulfide in vascular smooth muscle cells. Mol Pharmacol 2005; 68(6): 1757–1764.

48 Sun Y, Tang CS, Jin HF, Du JB. The vasorelaxing effect of hydrogen sulfide on isolated rat aortic rings versus pulmonary artery rings. Acta Pharmacol Sin 2011; 32(4): 456–464.

49 Li L, Whiteman M, Guan YY, Neo KL, Cheng Y, Lee SW, Zhao Y, Baskar R, Tan CH, Moore PK. Characterization of a novel, water-soluble hydrogen sulfide-releasing molecule (GYY4137): New insights into the biology of hydrogen sulfide. Circulation 2008; 117(18): 2351–2360.

50 Dellinger RP, Carlet JM, Masur H, Gerlach H, Calandra T, Cohen J, Gea-Banacloche J, Keh D, Marshall JC, Parker MM, Ramsay G, Zimmerman JL, Vincent JL, Levy MM. Surviving Sepsis Campaign guidelines for management of severe sepsis and septic shock. Intensive Care Med 2004; 30(4): 536–555.

51 Landry DW, Levin HR, Gallant EM, Ashton RC Jr, Seo S, D'Alessandro D, Oz MC, Oliver JA. Vasopressin deficiency contributes to the vasodilation of septic shock. Circulation 1997; 95(5): 1122–1125.

52 Landry DW, Levin HR, Gallant EM, Seo S, D'Alessandro D, Oz MC, Oliver JA. Vasopressin pressor hypersensitivity in

vasodilatory septic shock. Crit Care Med 1997; 25(8): 1279–1282.

53 Holmes CL, Walley KR, Chittock DR, Lehman T, Russell JA. The effects of vasopressin on hemodynamics and renal function in severe septic shock: a case series. Intensive Care Med 2001; 27(8): 1416–1421.

54 Shi W, Cui N, Shi Y, Zhang X, Yang Y, Jiang C. Arginine vasopressin inhibits Kir6.1/SUR2B channel and constricts the mesenteric artery via V1a receptor and protein kinase C. Am J Physiol Regul Integr Comp Physiol 2007; 293(1): R191–199.

55 Tan JH, Al Abed A, Brock JA. Inhibition of K_{ATP} channels in the rat tail artery by neurally released noradrenaline acting on postjunctional α2-adrenoceptors. J Physiol 2007; 581(2): 757–765.

56 Park WS, Ko EA, Han J, Kim N, Earm YE. Endothelin-1 acts via protein kinase C to block K_{ATP} channels in rabbit coronary and pulmonary arterial smooth muscle cells. J Cardiovasc Pharmacol 2005; 45(2): 99–108.

57 Kubo M, Quayle JM, Standen NB. Angiotensin II inhibition of ATP-sensitive K^+ currents in rat arterial smooth muscle cells through protein kinase C. J Physiol 1997; 503(Pt 3): 489–496.

58 Bonev AD, Nelson MT. Vasoconstrictors inhibit ATP-sensitive K^+ channels in arterial smooth muscle through protein kinase C. J Gen Physiol 1996; 108(4): 315–323.

59 Tanaka E, Mori H, Chujo M, Yamakawa A, Mohammed MU, Shinozaki Y, Tobita K, Sekka T, Ito K, Nakazawa H. Coronary vasoconstrictive effects of neuropeptide Y and their modulation by the ATP-sensitive potassium channel in anesthetized dogs. J Am Coll Cardiol 1997; 29(6): 1380–1389.

60 Kleppisch T, Nelson MT. Adenosine activates ATP-sensitive potassium channels in arterial myocytes via A2 receptors and cAMP-dependent protein kinase. Proc Natl Acad Sci U S A 1995; 92(26): 12441–12445.

61 Mutafova-Yambolieva VN, Keef KD. Adenosine-induced hyperpolarization in guinea pig coronary artery involves A2b receptors and K_{ATP} channels. Am J Physiol 1997; 273(6 Pt 2): H2687–H2695.

62 Yang Y, Shi Y, Guo S, Zhang S, Cui N, Shi W, Zhu D, Jiang C. PKA-dependent activation of the vascular smooth muscle isoform of K_{ATP} channels by vasoactive intestinal polypeptide and its effect on relaxation of the mesenteric resistance artery. Biochim Biophys Acta 2008; 1778(1): 88–96.

63 Green BD, Hand KV, Dougan JE, McDonnell BM, Cassidy RS, Grieve DJ. GLP-1 and related peptides cause concentration-dependent relaxation of rat aorta through a pathway involving K_{ATP} and cAMP. Arch Biochem Biophys 2008; 478(2): 136–142.

64 Shi Y, Cui N, Shi W, Jiang C. A short motif in Kir6.1 consisting of four phosphorylation repeats underlies the vascular K_{ATP} channel inhibition by protein kinase C. J Biol Chem 2008; 283(5): 2488–2494.

65 Jiao J, Garg V, Yang B, Elton TS, Hu K. Protein kinase C-epsilon induces caveolin-dependent internalization of vascular adenosine 5'-triphosphate-sensitive K^+ channels. Hypertension 2008; 52(3): 499–506.

66 Haba M, Hatakeyama N, Kinoshita H, Teramae H, Azma T, Hatano Y, Matsuda N. The modulation of vascular ATP-sensitive K^+ channel function via the phosphatidylinositol 3-kinase-Akt pathway activated by phenylephrine. J Pharmacol Exp Ther 2010; 334(2): 673–678.

67 Kinoshita H, Matsuda N, Kaba H, Hatakeyama N, Azma T, Nakahata K, Kuroda Y, Tange K, Iranami H, Hatano Y. Roles of phosphatidylinositol 3-kinase-Akt and NADPH oxidase in adenosine 5'-triphosphate-sensitive K^+ channel function impaired by high glucose in the human artery. Hypertension 2008; 52(3): 507–513.

68 Quinn KV, Giblin JP, Tinker A. Multisite phosphorylation mechanism for protein kinase A activation of the smooth muscle ATP-sensitive K^+ channel. Circ Res 2004; 94(10): 1359–1366.

69 Shi Y, Wu Z, Cui N, Shi W, Yang Y, Zhang X, Rojas A, Ha BT, Jiang C. PKA phosphorylation of SUR2B subunit underscores vascular K_{ATP} channel activation by beta-adrenergic receptors. Am J Physiol Regul Integr Comp Physiol 2007; 293(3): R1205–R1214.

70 Purves GI, Kamishima T, Davies LM, Quayle JM, Dart C. Exchange protein activated by cAMP (Epac) mediates cAMP-dependent but protein kinase A-insensitive modulation of vascular ATP-sensitive potassium channels. J Physiol 2009; 587(Pt 14): 3639–3650.

71 Orie NN, Thomas AM, Perrino BA, Tinker A, Clapp LH. Ca^{2+}/calcineurin regulation of cloned vascular K_{ATP} channels: crosstalk with the protein kinase A pathway. Br J Pharmacol 2009; 157(4): 554–564.

72 Maritim AC, Sanders RA, Watkins JB 3rd. Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol 2003; 17(1): 24–38.

73 Ross J, Armstead WM. Differential role of PTK and ERK MAPK in superoxide impairment of K_{ATP} and K_{Ca} channel cerebrovasodilation. Am J Physiol Regul Integr Comp Physiol 2003; 285(1): R149–R154.

74 Yang Y, Shi W, Cui N, Wu Z, Jiang C. Oxidative stress inhibits vascular K_{ATP} channels by *S*-glutathionylation. J Biol Chem 2010; 285(49): 38641–38648.

75 Erdos B, Simandle SA, Snipes JA, Miller AW, Busija DW. Potassium channel dysfunction in cerebral arteries of insulin-resistant rats is mediated by reactive oxygen species. Stroke 2004; 35(4): 964–969.

76 Miura H, Wachtel RE, Loberiza FR Jr, Saito T, Miura M,

Nicolosi AC, Gutterman DD. Diabetes mellitus impairs vasodilation to hypoxia in human coronary arterioles: reduced activity of ATP-sensitive potassium channels. Circ Res 2003; 92(2): 151–158.

77 Yang Y, Shi W, Chen X, Cui N, Konduru AS, Shi Y, Trower TC, Zhang S, Jiang C. Molecular basis and structural insight of vascular K_{ATP} channel gating by S-glutathionylation. J Biol Chem 2011; 286(11): 9298–9307.

78 Ren Y, Xu X, Wang X. Altered mRNA expression of ATP-sensitive and inward rectifier potassium channel subunits in streptozotocin-induced diabetic rat heart and aorta. J Pharmacol Sci 2003; 93(4): 478–483.

79 Fan LH, Tian HY, Wang J, Huo JH, Hu Z, Ma AQ, Cao YX. Downregulation of Kir6.1/SUR2B channels in the obese rat aorta. Nutrition 2009; 25(3): 359–363.

80 Chatterjee S, Al-Mehdi AB, Levitan I, Stevens T, Fisher AB. Shear stress increases expression of a K_{ATP} channel in rat and bovine pulmonary vascular endothelial cells. Am J Physiol Cell Physiol 2003; 285(4): C959–C967.

81 Taguchi H, Heistad DD, Kitazono T, Faraci FM. ATP-sensitive K^+ channels mediate dilatation of cerebral arterioles during hypoxia. Circ Res 1994; 74(5): 1005–1008.

82 Xiao D, Longo LD, Zhang L. Role of K_{ATP} and L-type Ca^{2+} channel activities in regulation of ovine uterine vascular contractility: effect of pregnancy and chronic hypoxia. Am J Obstet Gynecol 2010; 203(6): 596e6–596e12.

83 Raeis V, Philip-Couderc P, Roatti A, Habre W, Sierra J, Kalangos A, Beghetti M, Baertschi AJ. Central venous hypoxemia is a determinant of human atrial ATP-sensitive potassium channel expression: evidence for a novel hypoxia-inducible factor 1α-Forkhead box class O signaling pathway. Hypertension 2010; 55(5): 1186–1192.

84 Vanelli G, Hussain SN, Aguggini G. Glibenclamide, a blocker of ATP-sensitive potassium channels, reverses endotoxin-induced hypotension in pig. Exp Physiol 1995; 80(1): 167–170.

85 Landry DW, Oliver JA. The ATP-sensitive K^+ channel mediates hypotension in endotoxemia and hypoxic lactic acidosis in dog. J Clin Invest 1992; 89(6): 2071–2074.

86 Kovalev H, Quayle JM, Kamishima T, Lodwick D. Molecular analysis of the subtype-selective inhibition of cloned K_{ATP} channels by PNU-37883A. Br J Pharmacol 2004; 141(5): 867–873.

87 O'Brien AJ, Thakur G, Buckley JF, Singer M, Clapp LH. The pore-forming subunit of the K_{ATP} channel is an important molecular target for LPS-induced vascular hyporeactivity *in vitro*. Br J Pharmacol 2005; 144(3): 367–375.

88 Czaika G, Gingras Y, Zhu E, Comtois AS. Induction of the ATP-sensitive potassium (uK_{ATP}-1) channel by endotoxemia. Muscle Nerve 2000; 23(6): 967–969.

89 Jin X, Malykhina AP, Lupu F, Akbarali HI. Altered gene expression and increased bursting activity of colonic smooth muscle ATP-sensitive K^+ channels in experimental colitis. Am J Physiol Gastrointest Liver Physiol 2004; 287(1): G274–G285.

90 Shi W, Cui N, Wu Z, Yang Y, Zhang S, Gai H, Zhu D, Jiang C. Lipopolysaccharides up-regulate Kir6.1/SUR2B channel expression and enhance vascular K_{ATP} channel activity via NF-κB-dependent signaling. J Biol Chem 2010; 285(5): 3021–3029.

91 Collin S, Sennoun N, Dron AG, de la Bourdonnaye M, Montemont C, Asfar P, Lacolley P, Meziani F, Levy B. Vascular ATP-sensitive potassium channels are over-expressed and partially regulated by nitric oxide in experimental septic shock. Intensive Care Med 2011; 37(5): 861–869.

92 Yu L, Jin X, Yang Y, Cui N, Jiang C. Rosiglitazone inhibits vascular K_{ATP} channels and coronary vasodilation produced by isoproterenol. Br J Pharmacol 2011; 164(8): 2064–2072.

93 Aziz Q, Thomas A, Khambra T, Tinker A. Phenformin has a direct inhibitory effect on the ATP-sensitive potassium channel. Eur J Pharmacol 2010; 634(1–3): 26–32.

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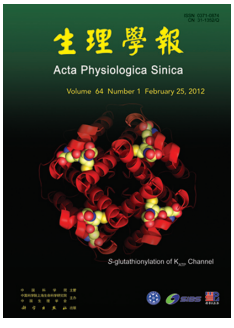

About Cover: The picture shows a computer model of the structure of four Kir6.1 pore-forming domains of ATP-sensitive potassium (K_{ATP}) channel bound with four glutathione molecules viewed from the intercellular side of the plasma membrane. This kind of protein modulation is known as *S*-glutathionylation. SHI Wei-Wei *et al*. reviewed the vascular K_{ATP} channel regulation under physiological and pathophysiological conditions (see page 1–13). The Kir6.1 pore-forming domains are colored red, resembling the shape of a Chinese knot. Cover credit: YANG Yang, Yale University School of Medicine.