REVIEW ARTICLE

Inwardly rectifying potassium channel 5.1: Structure, function, and possible roles in diseases

Junhui Zhang a,b,f,1, Jian Han c,1, Lingfei Li d, Qiong Zhang a,b, Yanhai Feng a,b, Youzhao Jiang e, Fang Deng f, Yuping Zhang f, Qinan Wu g, Bing Chen f,**, Jiongyu Hu a,b,f,*

a Institute of Burn Research, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing, 400038, PR China
b State Key Laboratory of Trauma, Burns and Combined Injury, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing, 400038, PR China
c Department of Obstetrics and Gynecology, Daping Hospital, Third Military Medical University (Army Medical University), Chongqing, 400042, PR China
d Department of Dermatology, Daping Hospital, Third Military Medical University (Army Medical University), Chongqing, 400042, PR China
e Department of Endocrinology, People’s Hospital of Banan District, Chongqing, 401320, PR China
f Department of Endocrinology, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing, 400038, PR China
g Department of Endocrinology, Chongqing Cancer Hospital (Chongqing University Cancer Hospital), Chongqing, 40030, PR China

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Abstract
Inwardly rectifying potassium (Kir) channels make it easier for K⁺ to enter into a cell and subsequently regulate cellular biological functions. Kir5.1 (encoded by KCNJ16) alone can form a homotetramer and can form heterotetramers with Kir4.1 (encoded by KCNJ10) or Kir4.2 (encoded by KCNJ15). In most cases, homomeric Kir5.1 is non-functional, while heteromeric Kir5.1 on the cell membrane contributes to the inward flow of K⁺ ions, which can be regulated by intracellular pH and a variety of signaling mechanisms. In the form of a

* Corresponding author. Endocrinology Department, State Key Laboratory of Trauma, Burns and Combined Injury, Southwest Hospital, Third Military Medical University (Army Medical University), Gaotanyan Street, Shapingba District, Chongqing 400038, China.
** Corresponding author. Department of Endocrinology, Southwest Hospital, Third Military Medical University (Army Medical University), Gaotanyan Street, Shapingba District, Chongqing, 400038, China.
E-mail addresses: xnyychenbing@163.com (B. Chen), jiongyuhu@163.com (J. Hu).
1 Peer review under responsibility of Chongqing Medical University.
1 These authors contributed equally to this work.

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Introduction

It is well known that maintaining an electrochemical imbalance across the plasma membrane is an essential cellular biological process. A large number of integral membrane proteins play roles in facilitating ion transport across membranes, and among these, the family of inwardly rectifying potassium (Kir) channels is expressed in almost all human cells and plays critical roles in the regulation of potassium balance, maintenance of negative resting potential, regulation of cell volume, clearance of extracellular glutamate, regulation of myelination and control of cellular excitability.

Kir channels were first verified as anomalous rectifier K⁺ current channels. They make it easier for K⁺ to enter into rather than exit a cell; i.e., instead of conducting K⁺ ions by depolarization, as is the case of other K⁺ channels, Kir channels transport K⁺ ions on the basis of hyperpolarization. Kir channels have multiple physiological functions, depending on their type and location, and their activities are controlled by a variety of mediators, such as binding proteins, phospholipids and ions. Kir channel subunits contain two transmembrane domains linked with the characteristic K⁺-selective pore sequence, and they can be assembled into homotetramers or heterotetramers. Since the first discovery of Kir1.1 in 1993, approximately fifteen distinct clones have been identified, and they can be divided into seven major subfamilies (Kir1.x–Kir7.x, where x is the number of each member) and four functional subgroups: ATP-sensitive K⁺-transport channels and G protein-gated Kir channels. In general, their physiological roles have either been established or proposed.

However, the Kir5.x family is an exception. To date, Kir5.1 (encoded by KCNJ16) is the only identified member of this family. As a K⁺-transport channel, Kir5.1 mRNA has been found in the brain region, cochlea, kidney, thyroid, stomach, liver, pancreas, adrenal gland, spleen, testis, and parathyroid gland. With the increase in the number of studies into the roles of Kir channels, researchers are paying more attention to the pathophysiological functions of Kir5.1. This minireview aims to provide an overview regarding the functions of Kir5.1 and its potential roles in diseases.

Basic molecular structure and primary function of Kir5.1

The primary structure of Kir5.1 is common to all Kir channels (Fig. 1A). It contains a common motif of two putative transmembrane domains (TM1 and TM2) connected with an extracellular pore-forming region (H5) and cytoplasmic amino (NH₂)- and carboxy (COOH)-terminal domains. The H5 region functions as an “ion-selectivity filter” that shares the characteristic sequence T-X-G-Y(F)-G with other K⁺-selective ion channels. The NH₂ and COOH termini are considered gating regulators, and the C-terminal cytosolic domain, located downstream of the H5 region, is rich in β-sheets and provides binding sites where multiple intracellular signaling regulators interact (Fig. 1B). These binding sites are highly conserved and are important for inward rectification.

Commonly, four subunits with two TM domains each are necessary for the formation of a functional Kir channel in a tetrameric complex, which can be either homomeric or heteromeric. Generally, heteromerized Kir channels are found in members of the same subfamily; for instance, Kir3.1 can associate with other Kir3.x subfamily members, such as Kir3.2 or Kir3.4. An exception is Kir4.1, which can assemble with Kir5.1. Coexpression studies have demonstrated that Kir5.1 forms functional heteromeric complexes with Kir4.1 (encoded by KCNJ10) to construct novel Kir4.1/Kir5.1 channels. Homomeric Kir5.1 expressed in the heterologous expression systems, unlike the heteromeric Kir4.1/Kir5.1 complex that is stable and functional on the plasma membrane of various cells, was promptly exocytosed and internalized across the membrane surface and was unable to yield any currents. In addition to heteromerization with Kir4.1, Kir5.1 may also form a heteromeric complex with Kir4.2 (encoded by KCNJ15), which is a close homolog of Kir4.1 and is widely distributed in the brain, kidney, lung, liver and pancreas. To date, no other channel has been demonstrated to form functional channels with Kir5.1. The mechanisms by which Kir4.1 and Kir4.2 rescue the function of Kir5.1 remain unknown.

The Kir4.1/Kir5.1 heterotetramer acts as the predominant potassium channel in distal nephron segments

K⁺ channels play important roles in regulating renal ion transportation directly by providing a secretory pathway for K⁺ or indirectly by modulating K⁺ recycling across the plasma membrane and controlling the membrane potential. Heteromeric Kir4.1/Kir5.1 channels are widely found in the principal cells of the cortical thick ascending limb (TAL), distal convoluted tubule (DCT), and cortical collecting duct (CCD). The basolateral location of...
Kir4.1/Kir5.1 channels indicates their role in the maintenance of basolateral membrane potential and in the "recycling" of the K⁺ that enters a cell via basolateral Na⁺/K⁺ ATPase. It has been shown that the recycling of K⁺ is also critical for sustained transepithelial Na⁺ reabsorption. Recent studies have demonstrated that rare homozygous missense mutations of the human KCNJ10 gene (encoding the Kir4.1 subunit) cause a rare disorder in which patients experience neurological and renal symptoms, namely, seizures, sensorineural deafness, ataxia, mental retardation, electrolyte imbalance (SeSAME)/epilepsy, ataxia, sensorineural deafness, and renal tubulopathy (EAST) syndrome. Loss-of-function mutations in Kir4.1 in the kidney have been found to inhibit the function of both homomeric Kir4.1/Kir4.1 channels and heteromeric Kir4.1/Kir5.1 channels and thus lead to a significant decrease in salt reuptake from the DCT and an increase in downstream K⁺ and H⁺ secretion. These mutations cause a Gitelman-like syndrome that is characterized by salt wasting, hypomagnesaemia, hypocaliuria and hypokalemic metabolic alkalosis.

Kir5.1-knockout mice did not recapitulate the phenotypes of SeSAME/EAST syndrome, except for hypokalemia. Deletion of the KCNJ16 gene (encoding the Kir5.1 subunit) also led to a severe renal phenotype in adult mice, manifesting by K⁺ loss, hyperchloremia, hypocaliuria and metabolic acidosis. Kir5.1 knockout disrupted the formation of heteromeric Kir4.1/Kir5.1 channels but not homomeric Kir4.1/Kir4.1 channels. The unexpected increase in basolateral K⁺ conductance in the DCT indicated that Kir4.1 could compensate for the loss of Kir5.1 and sustain ion transport in the DCT. Thus, the potential mechanism underlying the phenotypic distinction between KCNJ10 deletion and KCNJ16 deletion may be that the KCNJ16 deletion induces the constitutively high activity of homomeric Kir4.1/Kir4.1 channels that results in the elevation of K⁺ conductance in the basolateral membrane, enhances basolateral Cl⁻ outflow, leads to hyperchloremia and acidosis, elevates the activities of Na⁺-K⁺-ATPase and NCC (sodium chloride transporter), and consequently promotes overall salt reabsorption by the DCT.

Mechanisms involved in the regulation of heteromeric Kir5.1

A significant difference among homomeric Kir4.1, Kir4.2 and heteromeric Kir4.X/5.1 channels is their distinct sensitivity to alterations in pHᵢ. Over the physiological range of pHᵢ, between 6.5 and 8.0, the activity of homomeric Kir4.1 channels can be remarkably impaired by acidification with a pKₐ = 6.0. Under the same pHᵢ range, the activity of heteromeric channels is significantly downregulated by slight intracellular acidification and dramatically enhanced by alkalinization with a pKₐ = 7.5. Kir4.2 is more sensitive to pHᵢ (pKₐ ≈ 7.1), and Kir5.1 has similar effects on the pHᵢ sensitivity of Kir4.2 when they are coexpressed (pKₐ ≈ 7.6). Their pHᵢ sensitivity strongly indicates that they are pHₐ sensors and mediators in pHₐ-dependent regulation of renal ion transport. However, the underlying mechanism by which Kir5.1 modulates the pHₐ sensitivity of Kir4.x remains elusive.

Among the emerging studies on Kir4.1/Kir5.1, some studies focused on expression regulation. It was recently shown that dopamine, an important regulator of systemic blood pressure, reversibly downregulated the activity and opening probability (Pₒ) of both basolateral Kir4.1/Kir5.1 and Kir4.1/Kir4.1 channels in freshly isolated murine CCD cells. This effect involves the activation of D₂-like dopamine receptors and subsequent stimulation of PKC. It was also revealed that insulin and insulin-like growth factor-1 (IGF-1) increased the opening probability of Kir4.1/Kir5.1
in a phosphatidylinositol 3-kinase (P3K)-dependent manner, resulting in significant hyperpolarization of the basolateral membrane. In addition, the application of norepinephrine increased the basolateral Kir4.1/Kir5.1 heterotetramer activity level in the DCT, which could be mimicked by a cAMP analog but was abolished by protein kinase A (PKA) inhibition. Moreover, bradykinin (BK) was observed to inhibit the activity of the basolateral Kir4.1/Kir5.1 channels in a PKC-dependent manner. The specific antagonist of bradykinin B2 receptor (BK2R), but not an antagonist of BK1R, abolished the effect of BK on the basolateral Kir4.1/Kir5.1 channels in the DCT. Thus, BK2R plays a role in the tonic regulation of the basolateral Kir4.1/Kir5.1 channels in the DCT.

It has been demonstrated that the pH sensitivity of Kir4.1/Kir5.1 heterotetramers is much greater than that of Kir4.1 homotetramers, thus, Kir5.1 may act as a regulator of Kir4.1 function. The amino acid sequence analysis of Kir5.1 indicates that Kir5.1 contains a phospho-threonine motif (TPVT) in its C-terminus (AA 249 to 252), which has been proven to bind to Nedd-4, an E3 ubiquitin ligase. This TPVT motif is highly preserved in Kir5.1 from humans to rats and mice. The notion that Kir5.1 may modulate the ubiquitination of Kir4.1 is supported by the finding that deficient Kir5.1 or Nedd-2 significantly decreases the expression of Kir4.1. The basolateral K+ currents in the DCT and Kir4.1 expression are both significantly increased in Kir5.1-knockout mice or in the kidney-specific Nedd4-2-deficient Kir5.1 or Nedd4-2 significantly increases the ubiquitination of Kir4.1 is supported by the finding that deficient Kir5.1 or Nedd-2 significantly decreases the expression of Kir4.1. The basolateral K+ currents in the DCT and Kir4.1 expression are both significantly increased in Kir5.1-knockout mice or in the kidney-specific Nedd4-2-knockout compared to the levels in the corresponding wild-type (WT) littermates. These observations indicate that Kir5.1 is a potential regulatory subunit for the function of Kir4.1/Kir5.1 heterotetramers.

Possible role of Kir5.1 in extrarenal diseases

**Kir 5.1 and chemosensory control of ventilation**

Ventilation is continuously adjusted by a neural network to maintain the homeostasis of blood gases and pH. Brainstem serotonin (5-HT) neurons play an important role in regulating ventilation and are stimulated by hypercapnic acidosis, the sensitivity of which elevates with increasing postnatal age. A study of transcriptomes identified age-dependent shifts in multiple ion channels in rat 5-HT neurons, including but not limited to, pH-sensitive potassium ion channel genes, such as KCNJ10 (encoding the Kir4.1 protein) and KCNJ16 (encoding the Kir5.1 protein). Immunofluorescence imaging confirmed that the Kir5.1 protein is colocalized to 5-HT neurons, whereas the Kir4.1 protein is not detected. These data indicate that Kir5.1 may regulate cellular CO2/pH chemosensitivity in brainstem 5-HT neurons. Recently, Kir5.1-knockout mice (Kir5.1−/−) were used to explore the role of Kir5.1 in the chemosensory control of breathing. It was reported that deletion of Kir5.1 in these mice resulted in a clear respiratory phenotype, but the loss of Kir5.1 did not directly affect the function of either central or peripheral respiratory chemoreceptors. Despite profound metabolic acidosis, ventilation at rest (normoxia–normocapnia) and under hypoxic hypercapnia was similar in the wild-type and Kir5.1−/− mice. When exposed to hypoxia and normoxic hypercapnia, the wild-type mice presented with an increased rate and depth of breathing, while the ventilatory responses were remarkably reduced in the Kir5.1−/− mice because the transmission of signals from the peripheral chemoreceptors to the CNS was compromised. More recent data from rats also demonstrated that Kir5.1 deletion reduced ventilatory responses to hypercapnia and hypoxia. Loss of Kir5.1 resulted in a combination of renal and respiratory phenotypes, including chronic hyperchloremic metabolic acidosis and hyperventilation. Partial correction of the arterial pH in the Kir5.1−/− rats attenuated the increase in ventilation when the rats were breathing room air and led to a further reduction in ventilatory sensitivity induced by hypercapnic acidosis. These observations suggest that Kir5.1 is an important regulator of acute (respiratory) and chronic (renal) pH, including the hypcapnic ventilatory response, and significantly contributes to hypoxic ventilatory chemoreflexes.

**Kir 5.1 and hearing impairment**

There are two extracellular fluids in the cochlea of the inner ear, i.e., perilymph and endolymph. The endolymph contains ~150 mM K+ and has a highly positive potential of ~+80 mV, compared with either blood or perilymph levels, and is named the "endocochlear potential". The unique ionic and voltage environment in the endolymph is critical for maintaining proper hearing.

Kir4.1 and Kir5.1 are both expressed in the cochlea, but their distributions are different. Kir4.1 is mainly expressed at the apical membrane of the intermediate cells in the stria, while Kir5.1 is specifically expressed in the cytoplasm of the fibrocyte in the spiral ligament. It is likely that Kir4.1 has a large K+ diffusion potential across this plasma membrane and constitutes an important fraction of the endocochlear potential. Further results demonstrate that Kir4.1 knockout induces deafness in mice and leads to an ~50% reduction in [K+] in the endolymph. The endocochlear potential of Kir4.1-knockout mice was found to be almost 0 mV. The Kir5.1 homomeric channels seem to be mainly localized in the intracellular compartments of fibrocytes. Both the mRNA and protein levels of Kir5.1 in the cochlea in aging C57BL/6J mice were downregulated compared with those in young C57BL/6J mice. Interestingly, perilymphatic perfusion of Ba2+, which blocks Kir channels, including Kir4.1 and Kir4.2, slightly elevates the endocochlear potential. It is likely that a small population of Kir5.1 homomers might negatively regulate K+ circulation across the plasma membrane of the spiral ligament.

**Kir5.1 and cardiovascular disease**

Brugada syndrome (BrS) is one of the ion channelopathies associated with sudden cardiac death (SCD). Two BrS patients presenting with SCD were found to have compound mutations, such as KCNJ16:Ser261Gly, which were predicted to be harmful variants. The KCNJ16 mutation showed 89% conservation in 46 species, suggesting that it was not a mutation hotspot. Its deregulation may lead to an
imbalance in membrane potential, which is associated with increased arrhythmogenesis.

With KCNJ16 knockout in Dahl salt-sensitive (SS) rats (SSKCNJ16/−/−), Kir5.1 was recently proven to play an important role in the control of blood pressure under conditions of salt-induced hypertension. The rats exhibited reduced blood pressure and hypokalemia. When fed a high-salt diet (4% NaCl), they experienced 100% mortality within a few days that was triggered by salt wasting and severe hypokalemia. Both benzamil and supplementation with a high-salt diet with increased potassium (2% KCl) prevented the mortality of the SSKCNJ16/−/− rats. These observations indicated that KCNJ16 is essential for the beneficial effects of a high potassium diet. In addition, these results suggested that Kir5.1 may be a potential target of hypertension treatment.

Kir5.1 and cancer

The roles of potassium channels in cell proliferation, cell motility and angiogenesis have only been assessed recently. Because potassium channels are accessible proteins and their pharmacology is well characterized, interest has been increasingly focused on developing potassium channels as therapeutic targets for cancer treatment. Although rare, the possible correlation between Kir5.1 and cancer has been reported in recent years. For instance, the results of the microarray transcriptome analysis showed that KCNJ16 was significantly upregulated in parathyroid carcinoma, a rare malignant neoplasm derived from the parenchymal cells of the parathyroid. To our knowledge, this is the first study to associate KCNJ16 with cancer. Then, using gene expression profile, KCNJ16 (downregulated 3.4-fold) was identified as one of the differentially expressed genes in a cirrhosis group and a hepatocellular carcinoma group, indicating that it might be a biomarker for the prediction of HBV-related cirrhosis progression to liver carcinoma. In anaplastic thyroid carcinoma, KCNJ16 was indicated as one of the differentially expressed genes (DEGs) and was validated to be significantly downregulated using real-time PCR. K+ channels play important roles in the endocrine and exocrine functions of the pancreas. The results from the screening of a GEO data set revealed that KCNJ16 is downregulated in pancreatic ductal adenocarcinoma (PDAC). Moreover, frequent mutations of KCNJ16 were observed in both spontaneous and ionizing radiation (IR)-associated lymphomas. Most of the above-mentioned data come from bioinformatics analysis and prediction. To date, there is a lack of conclusive evidence to prove the significance of its expression in tumor cells, but these data at least suggest that KCNJ16 may play a role in tumorigenesis and development. The complex regulatory mechanisms of KCNJ16 in cancer progression remain largely unknown and need to be explored in the future.

Concluding remarks

In the basolateral membrane of DCT, Kir4.1 expressed with Kir5.1 appears to be relevant for salt transportation and the adaptation of DCT to plasma K+ levels, which can be referred to as K+ sensing. Loss-of-function mutations in KCNJ10 (Kir4.1) are known to result in a complicated disorder with renal salt-wasting tubulopathy and alkalosis, namely, EAST/SeSAME syndrome. Deletion of Kir5.1 leads to hypokalemia, metabolic acidosis and hypercalciuria in mice, which indicates its role in renal transport. It is widely known that Kir5.1 commonly interacts with Kir4.1 or Kir4.2 to constitute heterotetramers and regulate ion transport. However, it is not clear whether Kir5.1 can function alone under some conditions, especially in cases in which neither Kir4.1 or Kir4.2 is not expressed. Moreover, this review sheds some light on the role of Kir5.1 in some extrarenal diseases, such as hypercapnic acidosis, cardiovascular disease, and several cancers. Further molecular and animal experiments clearly remain to be completed to increase our knowledge about the precise role of Kir5.1 in human diseases, which may be helpful for developing novel, specific pharmacological tools for the treatment of renal and other diseases.

Authors contribution

Jiongyu Hu and Bing Chen conceived the review, participated in its design and helped to draft the manuscript. Junhui Zhang and Jian Han performed the material collection, data analysis, and manuscript writing. Lingfei Li, Qiong Zhang, Yanhai Feng, Youzhao Jiang, Fang Deng, Yuping Zhang, and Qinan Wu revised the manuscript. All authors read and approved the final manuscript.

Conflict of Interests

The authors have no conflicts of interest to declare.

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