Emerging Concepts

The Emerging Structural Pharmacology of ATP-Sensitive Potassium Channels

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ABSTRACT
ATP-sensitive potassium channels (KATP) are energy sensors that participate in a range of physiologic processes. These channels are also clinically validated drug targets. For decades, KATP inhibitors have been prescribed for diabetes and KATP activators have been used for the treatment of hypoglycemia, hypertension, and hair loss. In this Emerging Concepts article, we highlight our current knowledge about the drug binding modes observed using cryogenic electron microscopy techniques. The inhibitors and activators bind to two distinct sites in the transmembrane domain of the sulfonylurea receptor (SUR) subunit. We also discuss the possible mechanism of how these drugs allosterically modulate the dimerization of SUR nucleotide-binding domains (NBDs) and thus KATP channel activity.

SIGNIFICANCE STATEMENT
ATP-sensitive potassium channels (KATP) are fundamental to energy homeostasis, and they participate in many vital physiological processes. Both KATP inhibitors (insulin secretagogues) and KATP activators are broadly used clinically for the treatment of related diseases. Recent cryogenic electron microscopy studies allow us to understand the emerging concept of KATP structural pharmacology.

The Emerging Concept of ATP-Sensitive Potassium Channel Structural Pharmacology

The potassium currents passing through ATP-sensitive potassium channels (KATP) are inhibited by intracellular ATP and activated by intracellular Mg-ADP (Nichols, 2006). As such, KATP channels tune their potassium conductance according to changes in the intracellular ATP/ADP ratio of the cell. KATP channels play essential roles in several important physiologic processes, including insulin secretion and vasodilation (Ashcroft, 2006; Flagg et al., 2010). The genetic mutations of KATP channels lead to human diseases such as neonatal diabetes and cardiovascular diseases (Ashcroft, 2006; Flagg et al., 2010). The work is supported by grants from the National Natural Science Foundation of China [Grant 91957201], [Grant 31870833], and [Grant 31821091] (to L.C.) and Center for Life Sciences.

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Abbreviations: cryo-EM, cryogenic electron microscopy; CTD, cytoplasmic domain; GBM, glibenclamide; IS, insulin secretagogue; ISBS, insulin secretagogue binding site; KATP, ATP-sensitive potassium channel; KCO, KATP opener; KOS, KATP opener binding site; Kir, inward-rectifier potassium channel; KNtp, Kir6 N-terminal peptide; MTG, mitiglinide; NBD, nucleotide-binding domain; RPG, repaglinide; SUR, sulfonylurea receptor; TMD, transmembrane domain.

KATP channels are assembled from four pore-forming inward-rectifier potassium channel 6 (Kir6) subunits and four regulatory sulfonylurea receptor (SUR) subunits (Fig. 1, A–C). The Kir6 subunit in the center has the inhibitory nucleotide-binding site, whereas the SUR subunit in the peripheral harbors the activating Mg-nucleotide-binding site (Fig. 1, A–C). The interplay of these nucleotide-binding sites during the fluctuation of intracellular nucleotide concentrations shapes the overall KATP channel activities. In the past several decades, fruitful medicinal research on KATP channels has established that SUR proteins, either SUR1 or SUR2, are validated drug targets (Li et al., 2021). SUR proteins belong to the subfamily C of the ATP-binding cassette transporter (ABCC) subfamily of the ATP binding cassette (ABC) transporters and have a transmembrane domain 0 (TMD0), which is unique to certain ABCC family members. SUR proteins have a canonical structural core formed by TMD1-NBD1-TMD2-NBD2 (Attionumi et al., 2009; ter Beek et al., 2014) (Fig. 1D). The helices from transmembrane domain 1 (TMD1) and TMD2 are intertwined to form two structural halves. The first half consists of TM6–8, TM11 of TMD1, and TM15–16 of TMD2; and the remaining...
Fig. 1. The insulin secretagogue binding site of SUR (A) and (B). Bottom view (from the intracellular side) and side view of the KATP hetero-octamer in complex with RPG (PDB code: 6JB1; EMDB code: 9787). The Kir6, SUR TMD0, TMD1-NBD1 (nucleotide binding domain 1), TMD2-NBD2, RPG, and ATP are colored in cyan, orange, pink, blue, yellow, and brown, respectively. TMD (transmembrane domain) and NBD (nucleotide binding domain). (C) The cartoon topology of Kir6 and SUR. PH (pore helix), IH (interfacial helix), CTD (cytoplasmic domain), ABLOS (ATP binding loop of SUR), ICL (intracellular loop), ECL (extracellular loop). (D) Side view of the SUR1 ABC transporter module in the presence of RPG and ATP (PDB code: 6JB1). (E) The chemical structures of IS. The hydrophobic A (H-A) and hydrophobic B (H-B) sites are in the gray background and the polar site (P) is in pink background, respectively. The IS without available KATP complex structure are colored in gray. (F–K) Close-up view of the binding site of (F) GBM (PDB code: 5YW7), (H) RPG (PDB code: 6JB3), and (J) MTG (PDB code: 7WIT). SUR1 and ISs are colored in gray and yellow, respectively. Residues of SUR1 that interact with IS are shown as sticks and colored as Fig. 1E according to the binding sites. The SUR1-IS interactions analyzed by LigPlot. (G) GBM, (I) RPG, and (K) MTG.
helices in the transporter core form the second half. SUR proteins were found to have two major conformations: the NBD-separated inward-facing conformation represents an inactive state, whereas the NBD-dimerized occluded conformation is associated with K\textsubscript{ATP} activation (Wu et al., 2020). SUR subunits also dominate the pharmacological profile of K\textsubscript{ATP} channels. K\textsubscript{ATP} channels in pancreatic cells are mainly formed by the SUR1-Kir6.2 combination. SUR1 inhibitors can trigger depolarization of β cells and thus insulin secretion. They are used for the treatment of diabetes (Li et al., 2021) and are therefore named "insulin secretagogues" (designated IS). Conversely, SUR1 activators can inhibit insulin secretion and thus can treat certain hypoglycemia (Li et al., 2021). SUR2 is expressed in blood vessels, heart, and skeletal muscles. SUR2 activators are used for the treatment of hypertension and hair loss (Li et al., 2021). SUR activators are called "K\textsubscript{ATP} openers" (designated KCO). Recent revolutionary advances in single particle cryogenic electron microscopy (cryo-EM) allowed the visualization of membrane protein structures at near-atomic resolution (Kühlbrandt, 2014). Using this technique, we and others have experimentally determined the binding sites and poses of not only K\textsubscript{ATP} inhibitors but also K\textsubscript{ATP} activators inside the SUR proteins.

**The IS Binding Site of K\textsubscript{ATP} Channel**

Structurally, IS are classified into sulfonylureas (Sus) [such as glibenclamide (GBM)] and glinides [such as repaglinide (RPG) and mitiglinide (MTG)] (Gribble and Reimann, 2003; Winkler et al., 2007; Wu et al., 2020). Based on the structure-activity relationship of IS, researchers guessed that there were the “A” site and “B” site on SUR long before the structure determination of K\textsubscript{ATP} (Rufer et al., 1974; Brown and Foubister, 1984; Winkler et al., 2007). Up to today, high-resolution structures of K\textsubscript{ATP} have revealed the binding sites of three IS inside the central vestibule of SUR, including GBM, RPG, and MTG (Li et al., 2017; Martin et al., 2017a,b, 2019; Wu et al., 2018; Ding et al., 2019; Sung et al., 2021; Wang et al., 2022a) (Fig. 1). The binding of IS stabilizes SUR in the inward-facing state with two NBDs separated (Fig. 1D). These structures have depicted the IS binding site (ISBS) at near-atomic resolution and allowed us to analyze IS-SUR interactions in detail (Fig. 1, F–K). We conceptually divide the ISBS into three connecting regions according to the structures: the hydrophobic A site (H-A), the polar site (P), and the hydrophobic B site (H-B) (Fig. 1E). Based on the common pharmacophores, we also tentatively list and align several representative IS, the structures of which in complex with K\textsubscript{ATP} are not available yet (Grell et al., 1998; Hu et al., 1999; Reimann et al., 2001b; Vila-Carriles et al., 2007; Winkler et al., 2007; Basit et al., 2012) (Fig. 1B).

Intriguingly, all of these IS interact with the P site and the H-B site (Fig. 1E). The P site is formed by positively charged R1246, R1300, and polar N1245 of SUR1 and interacts with the electrostatically negative group of IS, such as the sulfonyl group of GBM, and the carboxyl group of RPG and MTG (Fig. 1, E–K). Mutations at the P site abolished the inhibition of GBM, RPG, and MTG (Ding et al., 2019; Wang et al., 2022a).

IS have diverse chemical groups at the H-A site, which is one of the key determinants for the subtype selectivity of IS. S1238 of SUR1 at the H-A site is replaced by a much larger tyrosine in SUR2. Therefore, IS with a bulkier group at the H-A site could be accommodated by SUR1 but might not bind SUR2 favorably, in agreement with the order of SUR1/SUR2 selectivity: MTG > GBM > RPG (Quast et al., 2004; Wang et al., 2022a).

The common hydrophobic phenyl group of IS is bound in the hydrophobic pocket formed by residues on the H-B site, including F433 and I381 (Fig. 1, E–K). A large hydrophobic cavity formed by residues from helices TM6–8 and TM11 (Y377, F433, L434, M441, L592, V596, and W1297) accommodates the chlorobenzamide group of GBM or the large hydrophobic group including the piperidinylphenyl moiety and the butyl carbamoyl-methyl moiety of RPG (Fig. 1). GBM and RPG make differential interactions with the H-B site. R306 on TM8 of the H-B site of SUR1 only forms polar interactions with the chloride atom of GBM but not with RPG (Fig. 1, E–K), which is consistent with the decreased potency of GBM but not RPG to the R306A mutant of SUR1 (Ding et al., 2019). This was later confirmed in SUR1/Kir6.2 K\textsubscript{ATP} in the presence of RPG and even SUR2b/Kir6.1 K\textsubscript{ATP} in the presence of GBM (Ding et al., 2019; Martin et al., 2019; Sung et al., 2021). Therefore, the H-B site is formed not only by the SUR subunits but also by the K\textsubscript{Ntp}, providing a plausible mechanism for the IS-enhanced inhibitory coupling between SUR and Kir6. However, due to the flexibility of K\textsubscript{Ntp}, the exact residues that interact with IS remain poorly defined.

**The KCO Binding Site of K\textsubscript{ATP} Channel**

KCO are chemically diverse and comprise distinct classes, including cyanoguanidines (such as pinacidil and P1075), benzopyrans (such as levcromakalim, abbreviated as Lev), thiadiazines (such as diazoxide and NN414), and others (Tusnady et al., 1997; Gilbert et al., 2000; Mannhold, 2004, 2006). Their exact binding sites on the SUR subunit were elusive despite the enlightening work on chimeric SUR and mutagenesis (D’hahan et al., 1999a,b; Udhe et al., 1999; Ashcroft and Gribble, 2000; Babenko et al., 2000; Moreau el al., 2000; Reimann et al., 2001a). To experimentally determine the KCO binding site, our laboratory has solved the structure of SUR2 in complex with SUR2-selective P1075 and Lev (Ding et al., 2022) and SUR1/Kir6.2 K\textsubscript{ATP} with SUR1-selective NN414 (Wang et al., 2022b), providing comprehensive views of KCO binding sites (KCOs) at near-atomic resolutions (Fig. 2). The binding of KCO synergizes with Mg-nucleotides to stabilize the SUR in the NBD-dimerized occluded conformation (Fig. 2A) in agreement with the fact that KCO bind to SUR with slower off-rates in the presence of Mg-nucleotides (Gribble et al., 2000). Despite the distinct chemical structures and selectivities of these three KCO, they all bind at a common KCOs in the middle of the transmembrane domain of SUR (Fig. 2A) in agreement with the competitive binding behavior of KCO (Bray and Quast, 1992). KCOs is embraced by TM10, TM11, TM12, TM13, TM14, and TM17 helices (Fig. 2, C, E, and G) and is about 20 Å away from the aforementioned ISBS (Ding et al., 2022). Based on the structures, we define the KCOs as three connecting regions on SUR: the A site, the polar site (P site), and the B site (Fig. 2B).
Cry-EM Reveals K<sub>ATP</sub> Drug Binding Sites

Two residues in the P site, a His (H584 in SUR1 and H576 in SUR2) and an Asp (D1031 in SUR1 and D1008 in SUR2), play critical roles in interacting with the central polar groups of KCO. H584 of SUR1 interacts with the sulfonamide group on the thiadiazine ring of NN414, and D1031 forms hydrogen bonds between its carboxyl group and the thiadiazine ring of NN414 (Fig. 2, C and D). H576 on TM11 interacts with the guanidine group of P1075. D1008 on TM12 forms hydrogen bonds between its carboxyl group and guanidine ring on the other chiral carbon of Lev and H576 interacts with the hydroxyl group and pyrrolidinone ring on the other chiral carbon of Lev (Fig. 2, G and H). Structural information is consistent with previous studies showing that NH groups of pyridylamino group of P1075 can act as a hydrogen-bond donor and make a crucial contribution toward K<sub>ATP</sub> activation (Manley and Quast, 1992); the substitution of pyrrolidinone of Lev changed drug potency significantly (Mannhold, 2006); the substitution of the methyl group on the thiadiazine rings by alkylamino group enhanced the potency of dioxide derivatives (Hansen, 2006). Mutation of these two residues greatly decreased the activation effect of both SUR1-selective opener NN414 (Wang et al., 2022b) and SUR2-selective KCO, including P1075 and Lev (Ding et al., 2022), emphasizing that the polar interactions between the P site of SUR and KCO are essential for channel activation.

The A site is formed by residues in TMD2, and these residues are not identical between SUR1 and SUR2 (Ding et al., 2022). I1004 and T1253 in SUR2 are L1027 and M1290 in SUR1, respectively. I1004 of TM12 and T1253 of TM17 of SUR2 are close to the cyano group and pyrrolidinone ring of P1075. Mutations of I1004L and T1253M introduce steric hindrance to the binding of P1075 (Ding et al., 2022). Rb<sup>+</sup> efflux assay showed that I1004L in SUR2B reduced the activating effect of P1075 significantly and that T1253M in SUR2B had a more profound effect (Ding et al., 2022). In the structure of SUR2B in complex with Lev, I1004 and T1253 are located close to the benzonitrile group and pyrrolidinone ring of Lev, respectively (Fig. 2 G and

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**Fig. 2.** The KCO binding sites of SUR. (A) Structure of SUR2B in complex with levcromakalim (Lev) and Mg-nucleotides. TMD1-NBD1, TMD2-NBD2, Lev, Mg-ATP, and Mg-ADP are colored in pink, blue, yellow, cyan, and orange, respectively. (B) Chemical structures of KCO. A site, P site, and B site are in gray, pink, and green background, respectively. KCO without available K<sub>ATP</sub> complex structure are colored in gray. C-D. A site, P site, and B site are in gray, pink, and green background, respectively. KCO without available K<sub>ATP</sub> complex structure are colored in gray. C-D. A site, P site, and B site are in gray, pink, and green background, respectively. KCO without available K<sub>ATP</sub> complex structure are colored in gray.
H). I1004L and T1253M mutations in SUR2B reduced the activating effect of Lev to a similar extent (Ding et al., 2022). M1290 at the A site of SUR1 interacts with the hydrophobic methyl cyclopropane group of NN414 (Fig. 2, C and D). This group is replaced by a smaller methyl group in diazoxide (Fig. 2B), which could not efficiently make hydrophobic interactions with M1290, partially explaining the higher potency of NN414 than diazoxide in the activation of the SUR1-containing K<sub>ATP</sub> channel (Dabrowski et al., 2003).

The B site is mainly formed by TMD1. P551, I552, and V555 on TM10, L580 on TM11, 11030 on TM12, and C1072 on TM13 of SUR1 form a hydrophobic pocket and embrace the chlorothiophene ring of NN414 (Fig. 2, C and D). The hydrophobic pocket of SUR2 is formed by I545 and V548 of TM10, V579 of TM11, and I1004 and I1007 of TM12. Dimethylpropyl group of P1075 and benzopyran group of Lev are inserted into the hydrophobic pocket of SUR2 (Fig. 2, E–H). Although residues that form the B and P sites are conserved between SUR1 and SUR2 subunits, the B and P sites of KCOS in SUR1 is enlarged compared with SUR2. The C<sub>2</sub> distance between H576 and D1008 is increased from 10.2 Å in SUR2 (Ding et al., 2022) (PDB ID: 7VL8) to 12.4 Å in SUR1 (Wang et al., 2022b) (PDB ID: 7W40) due to the conformational difference of their transmembrane helices.

**Future Efforts**

As described above, the currently available structures have uncovered the ISBS and KCOS on the SUR subunits, but the exact mechanisms of how IS and KCOS typically remain incompletely understood. Based on previous functional studies and available structures, IS likely inhibit K<sub>ATP</sub> through two interdependent mechanisms. First, IS wedge in the TMD to allosterically inhibit the dimerization of two NBDs. Second, IS facilitate the rotation of Kir6.2 N-terminal peptide (KNtp) to the central vestibule of SUR1, and KNtp further restrains the rotation of Kir6.2 cytoplasmic domain (CTD) to inhibit channel opening (Wu et al., 2018, 2020). Despite recent progress in the cryo-EM studies of K<sub>ATP</sub> with intact KNtp (Li et al., 2017; Martin et al., 2017a, 2019; Sung et al., 2021) or KNtp-SUR1 chimera (Ding et al., 2019), the exact residues on KNtp that make interactions with IS and SUR1 could not be explicitly identified in currently available maps because of their poor local map qualities due to the flexibility of KNtp. Similarly, the pharmacophores on IS that are responsible for KNtp binding are also poorly defined, although it is obvious that different IS use distinct chemical groups at H-B site for KNtp recruitment (Fig. 1E). These fundamental questions about the structural pharmacology of IS await further structural and functional investigation. This allosteric model for IS inhibition is also in agreement with the shortcoming of IS: some neonatal diabetes patients with Kir6.2 mutations are resistant to the sulfonylurea therapy (SU-insensitive mutants) (Pipatpolkai et al., 2020). This is likely because the Kir6.2 CTDs of these SU-insensitive mutants seldom sample the closed conformation that allows the insertion of KNtp into the SUR1 central vestibule. Therefore, drugs that directly inhibit the Kir6.2 channel would be a better choice to suppress the activity of these mutants. However, available small-molecule inhibitors of Kir6.2 such as imidazoline-type compound phenotolamine are low affinity, and their binding sites are unknown yet (Cui et al., 2021). Recently, a centipede toxin, SpTx-1, has been reported to inhibit the Kir6.2 channel with high potency (Ramu et al., 2018), and SpTx-1 could trigger insulin secretion and lower blood glucose levels in the diabetic mice model (Ramu et al., 2022). We believe further elucidation of the structural mechanism of Kir6.2-specific small molecules or SpTx-1 toxins might shed light on the development of novel anti-diabetic drugs targeting SU-insensitive K<sub>ATP</sub> Mutants.

The structures of SUR in complex with KCO reveal that KCO activate the channel by promoting SUR to adopt the occluded state, which is in synergy with Mg-ADP (Ding et al., 2022, Wang et al., 2022b). Recent structures of the pancreatic K<sub>ATP</sub> channel in the activated nonconductive state (radii of the ion permeation pathway at the constriction are smaller than the hydrated potassium ion 3.3 Å) provide hints of how the occluded SUR activates K<sub>ATP</sub> (Zhao and MacKinnon, 2021; Wang et al., 2022b): first, the KNtp is released from the central vestibule of SUR, alleviating its allosteric effects on the inhibition of Kir6; and second, the ATP binding loop of SUR (ABLOS) is away from Kir6, which decreases the affinity of K<sub>ATP</sub> for the inhibitory nucleotides. However, the exact activation mechanism of K<sub>ATP</sub> by the occluded SUR awaits the determination of K<sub>ATP</sub> structure in the fully open conformation.

Taken together, recent progress on the structural determination of K<sub>ATP</sub>-drug complexes shed light on the concept of K<sub>ATP</sub> pharmacology at near-atomic resolution and sets the foundation for future structure-based drug design and optimization. However, a complete mechanistic understanding of the K<sub>ATP</sub> pharmacology awaits further investigation.

**Authorship Contributions**

Wrote or contributed to the writing of the manuscript: Wu, Ding, Chen.

**References**


