The therapeutic agents that target ATP-sensitive potassium channels

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ATP-sensitive potassium (K_{ATP}) channels are a major drug target for the treatment of type-2 diabetes. K_{ATP} channels are ubiquitously expressed and link the metabolic state to electrical excitability. In pancreatic β-cells, K_{ATP} channels are crucial in the regulation of glucose-induced insulin secretion. Also, K_{ATP} channels are involved in the protection against neuronal seizures and ischaemic stress in the heart, brain and in the regulation of vascular smooth muscle tone. Functional K_{ATP} channels are hetero-octamers composed of two subunits, a pore forming Kir6, which is a member of the inwardly rectifying potassium channels family, and a regulatory sulphonylurea receptor (SUR). In response to nucleotides and pharmaceutical agonists and antagonists, SUR allosterically regulates channel gating. The allosteric communication pathways between these two heterologus proteins in K_{ATP} channels are still poorly understood. This review will highlight the therapeutic agents that target K_{ATP} channels and are used to treat diabetes and cardiovascular diseases.

Keywords: ATP-sensitive potassium channels, therapeutic agents, diabetes, cardiovascular, inwardly rectifying potassium channels, sulphonylurea receptor

Potassium channels play a significant role in shaping the excitability and firing patterns of cells (1). Potassium channels are divided into four major classes based on their structure: two, four, six and seven transmembrane-domain channels (2). There is large diversity within potassium channels, including calcium activated, voltage-gated, twin pore domain, and inwardly rectifying channel subtypes. Despite this large diversity, the members of the channel family have similarities, such as all of them having pore-lining P-loops with a consensus amino acid sequence (3).

A biophysical property of inwardly rectifying potassium (Kir) channels is that they conduct inward current more readily than outward current (4, 5). Kir channels are crucial for stabilizing the resting membrane potential and regulating excitability in many tissues (1, 4). This group consists of seven sub-families: Kir1 to Kir7. All members of the Kir channel

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family have the same basic structure, which consists of intracellular amino (N) and carboxyl (C) termini and two putative membrane spanning segments (M1 and M2) flanking a pore-forming P-loop and signature sequence (6–8).

THE ATP-SENSITIVE POTASSIUM CHANNELS

The Kir channel proteins that have been identified comprise between 360 to 500 amino acids in length. All Kir members are regulated by the membrane phospholipid, phosphatidylinositol 4,5-bisphosphate (PIP_2) and some are also modulated by other regulatory factors or ligands, such as ATP and G-proteins, bearing their common names, ATP-sensitive (K_{ATP}) and the G-protein-gated potassium channel (GRK) (8).

ATP-sensitive potassium (K_{ATP}) channels, which are unique among potassium channels, were first described by Akinori Noma in 1983 (9). K_{ATP} channels have been identified in other tissues, including pancreatic β-cells (10), skeletal muscle cells (11), neuronal cells (12) and smooth muscle cells (13).

The classification of K_{ATP} channels is mainly based on pharmacological criteria using the potassium channel openers (KCOs, agonists) or sulphonylureas (antagonists). K_{ATP} channels are identified by subunit combinations of sulphonylurea receptor (SUR), SUR1, SUR2A and SUR2B, and inward rectifier (Kir), Kir6.1, Kir6.2, in different tissue types (14, 15).

THE PORE-FORMING INWARD RECTIFIER POTASSIUM CHANNEL ISOFORMS 6 (KIR 6)

The Kir6 subfamily is a member of the Kir channel family (16) and comprises the pore forming component of the K_{ATP} channel (17). There are two Kir6 isoforms, Kir6.1 and Kir6.2 (18). Kir6.1 and Kir6.2 isoforms are highly homologous and share 71 % amino acid identity with each other (19). These two isoforms form functional K_{ATP} channels with sulphonylurea receptors (19).

SULPHONYLUREA RECEPTOR

Sulphonylurea receptor (SUR) polypeptides are members of the ATP-binding cassette protein of the ABCC/MRP family. Human ABC protein genes are classified into seven subfamilies: ABCA to ABCG according to their gene structure, sequence homology and phylogenetic relations (20). There are two genes encoding three isoforms of SUR: SUR1, which is encoded by the ABCC8 gene, and SUR2 encoded by the ABCC9 gene. The latter can be transcribed into two different isoforms, SUR2A and SUR2B (21). These two SUR2 variants differ solely in the carboxyl-terminal due to alternative 3´-exon usage (22). In general, all ABC proteins contain a minimum of four structural domains: two transmembrane domains (TMDs) containing 6 to 8 transmembrane helices each and two cytosolic nucleotide binding domains (NBDs), which are involved in nucleotide binding and hydrolysis (23). SUR subunits consist of 17 transmembrane polypeptide segments clustered into three transmembrane domains (TMD), named TMD0, TMD1 and TMD2. TMD0 consists of the first five transmembrane segments and TMD1 and TMD2 consist of six segments each. There are two cytoplasmic nucleotide binding domains in each subunit. The first nucleotide binding domain NBD1 is located between transmembrane segments 11 and 12 and the
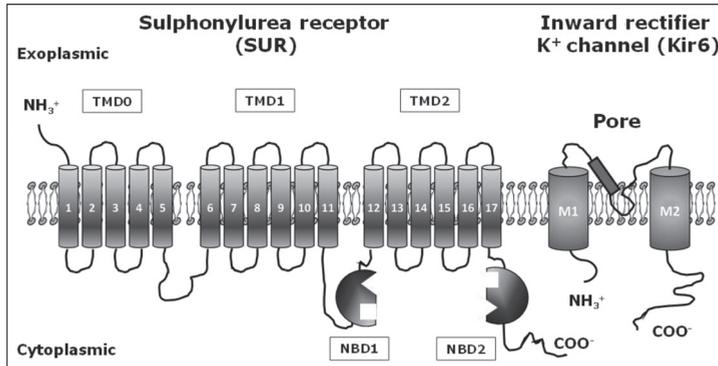
Fig. 1. Subunit structure of the ATP-sensitive potassium channel.

Schematic drawing showing the topology of the SUR and Kir6 subunits in K_{ATP} channels. The SUR subunit comprises the three transmembrane domains TMD0, TMD1 and TMD2, the two nucleotide binding domains NBD1 and NBD2, the extracellular N- and the intracellular C-termini and the pore-forming Kir subunit. Each Kir subunit consists of two transmembrane helices M1 and M2 and a pore-forming region containing the pore-helix, and intracellular N- and C-termini.

second, NBD2, is located beyond the last transmembrane segment number 17 and forms part of the C-terminal domain (21, 24). Both the sequence and the structure of NBDs are highly conserved across all prokaryotic and eukaryotic ABC proteins. Each contains a conserved Walker A (W_A) motif and a Walker B (W_B). These motifs catalyse ATP hydrolysis and are important for nucleotide regulation of the ABC proteins' functional activity (25).

As evidenced largely by Clement *et al.* (26), a functional K_{ATP} channel is an octameric complex composed of four SUR subunits and four Kir6 subunit, *i.e.*, a 4:4 stoichiometry ($Kir6_4/SUR_4$) (27). Binding of SUR to Kir subunits serves two purposes; first, to allow the translocation of the channel to the plasma membrane and, second, to contribute to the regulation of the channel by interaction between two subunits (28).

THE ROLE OF ATP-SENSITIVE POTASSIUM CHANNELS IN DIFFERENT TISSUES AND CELL TYPES

K_{ATP} channels couple cell metabolism to electrical activity in nerve, muscle and endocrine cells and play an important role in various cellular functions as sensors of intracellular ATP and ADP coupled to electrical function (18). K_{ATP} channels have important roles in many tissues under both physiological and pathological conditions (29).

The K_{ATP} channel has a key role in the physiology of many cells and defects in the channel itself or in its regulation such as in hyper/hypo-glycemia, ischaemia, hormone secretion and excitability of muscles/neurons causes human diseases (30).

K_{ATP} channels are crucial in the regulation of glucose-induced insulin secretion (18). In pancreatic β-cells, an increase in ATP/ADP ratio, generated by glucose uptake and metabolism, closes the K_{ATP} channels to elicit membrane depolarisation, calcium influx (opening of voltage-gated Ca^{2+} channels) and secretion of insulin, the primary hormone of glu-

cose homeostasis. In hyperglycaemia, increased transport of glucose into the β-cells occurs resulting in an elevated intracellular ATP, promoting closure of the K_{ATP} channels and membrane depolarization (31). This K_{ATP} channel mechanism can be mimicked by sulphonylurea drugs, for example, glibenclamide, which inhibits the K_{ATP} channel directly in pancreatic β-cells (32).

Potassium channels are critical to cardiac excitability because they play fundamental roles in setting the resting membrane potential, RMP, and in repolarisation of the action potential (AP). Under normal conditions, the cardiac sarcolemmal K_{ATP} channel is predominately closed (33). The channel activates during various forms of metabolic stress, including ischaemia, hypoxia, hyperglycemia, hypoglycaemia and inhibition of glycolysis and/or oxidative phosphorylation (34, 35).

In the vascular smooth muscle cells, K_{ATP} channels are thought to play important roles such as mediating the response of the vascular smooth muscle to a variety of pharmacological and endogenous vasodilators and also to changes in metabolic activity that can directly influence blood flow in various tissues (36). Most of the K_{ATP} channels in vascular muscle cells are rather insensitive to ATP and they are activated by nucleoside diphosphates and inhibited by glibenclamide (37).

K_{ATP} channels have been identified in various other tissues, including neurons, brain and skeletal muscle (30). K_{ATP} channels have been shown to be expressed in several regions of the brain, including the *substantia nigra* (38, 39) and in the hypothalamus (40). Evidence has shown that the K_{ATP} channels are also expressed in the *substantia nigra* area of the brain (41). It has been proposed that K_{ATP} channels play a role in the suppression of seizures in ATP-depleted conditions (42).

In the skeletal muscles, K_{ATP} channels have been identified by electrophysiological methods (43). The channels are mainly located in the plasma membrane, appear in all fibre types and are active in the resting human muscle. K_{ATP} channels located in the sarcolemma contribute significantly to membrane permeability in the resting human muscle and are important for the interstitial K^+ balance (44).

THE PHARMACOLOGY OF ATP-SENSITIVE POTASIUM CHANNELS

K_{ATP} channels are the major drug target and have the most therapeutic potential among potassium channels (3, 12, 45). Pharmacological treatment of a number of clinical conditions, such as angina and Type 2 diabetes, are targeted on the K_{ATP} channel, though in different ways. To treat angina, agonists such as potassium channel openers (KCOs), *e.g.*, nicorandil, are used to open the K_{ATP} channels in vascular smooth and cardiac muscles. In contrast to KCOs, antagonists such as sulphonylureas and related drugs are used to close K_{ATP} channels in pancreatic β-cells with, for example, tolbutamide or glibenclamide. Both types, agonists and antagonists, target the various SUR subunits to either open or close the channels.

It has been shown that co-expression of Kir6.2/SUR2A, Kir6.2/SUR2B, and Kir6.1/SUR2B reconstitutes the cardiac, smooth muscle and vascular smooth muscle K_{ATP} channels, respectively (46, 47). These channels have different sensitivities to ATP. They also show different responses to sulphonylurea drugs and potassium channel openers (19, 48, 49) permitting tissue selectivity in K_{ATP} channel directed therapies.

Table I. Classification of sulphonylureas, the ATP-sensitive potassium channel antagonists

Pharmacologic class			Mechanism of action	Clinical use
Sulphonylurea	First generation	*e.g.,* tolbutamide	blocking K_{ATP} channels in pancreatic β-cells prevents K^+ efflux and causes depolarization of the plasma membrane, then opens Ca^{2+} channels, causing influx of Ca^{2+} and insulin release	type 2 diabetes mellitus
	Second generation	*e.g.,* glibenclamide		
	Third generation	*e.g.,* glimepiride		

SULPHONYLUREAS

Sulphonylurea compounds were originally intended to be antimicrobial agents during World War II. At that time, it was observed that a common side effect was hypoglycaemia, now known to be due to the inhibition of pancreatic β-cell K_{ATP} channels. Today, sulphonylureas are used to treat type 2 diabetes (Table I) (50–52).

The target of sulphonylurea is the plasma membrane expressed K_{ATP} channel. At this site, sulphonylurea causes channel closure, which results in depolarization of the β-cell membrane and stimulation of insulin secretion (53). Kir6.2/SUR1 K_{ATP} channels are inhibited by glibenclamide (absolute inhibition constant K_i ~10 nmol L^{-1}). Kir6.2/SUR2A, Kir6.2/SUR2B and Kir6.1/SUR2B K_{ATP} channels are inhibited with K_i values in the low micromolar range (16, 19, 48). The binding is isoform dependent; the β-cell isoform SUR1 binds only tolbutamide and gliclazide and SUR2A and SUR2B bind all other types of sulphonylureas (51, 54–57).

As mentioned earlier, K_{ATP} channels are expressed ubiquitously however, sulphonylureas can cause undesired side effects since these drugs can cross-react with different K_{ATP} channel subtypes (58, 59). Chlorpropamide, acetohexamide, tolazamide and tolbutamide are the first-generation sulphonylureas and the action of these drugs is long lasting with considerable excretion in the urine (60). Hence, these drugs should be prescribed with cation for elderly patients with impaired kidney function (61).

Glyburide, glipizide and gliclazide are the second-generation sulphonylureas and they differ in pharmacokinetics and duration of action. It is worth mentioning that the second-generation sulfonylureas are more potent than the first-generation (60). A retrospective cohort study identified significant differences in ones risk of severe hypoglycaemia among users of individual sulphonylureas among older people (61). This study found relatively high incidence of hypoglycaemia in patients taking glyburide or chlorpropamide. Moreover, this study suggested a shorter-duration sulphonylureas such as glipizide elderly patients who cannot tolerate metformin as initial monotherapy (61).

Despite the differences in absorption and metabolism between the first- and second-generation sulphonylureas, they are equally effective in lowering blood glucose concentrations (62). Moreover, due to their structural characteristics, much lower doses of second-generation sulfonylureas are given than of the first generation (62). It has been reported

that in patients with type 2 diabetes, glimepiride caused low incidence of severe hypogly-caemia compared to glibenclamide (63). Also, the duration of glimepiride action is up to 24 hours (64).

Hirst *et al.* (65) reported that sulphonylurea monotherapy reduced HbA1c by an average of 1.5 % compared to that of placebo groups in a systematic review of double-blind randomized control trials. Sulphonylureas can lower the fasting blood glucose concentrations as extrapancreatic effects of these drugs. In other words, these drugs suppress the overnight hepatic glucose output (61, 66).

POTASSIUM CHANNEL OPENERS

Potassium channel openers are a chemically diverse group of agents, exemplified by pinacidil, levcromakalin, aprikalim, and nicorandil (Table II) (3, 12, 14, 25, 51, 67). These KCOs activate K_{ATP} channels (67–69). These agents possess high therapeutic potential in treating various clinical conditions such as hypertension, acute and chronic myocardial ischaemia, or congestive heart failure, and also in managing bronchial asthma, urinary incontinence and certain skeletal muscle myopathies (3, 45, 69). The effect of opening the K_{ATP} channel with KCOs is causing a shift of the membrane potential towards the reversal potential for potassium and, thereby, reducing it to the cellular electrical excitability.

The K_{ATP} channel subtype, which is stimulated by diazoxide, is Kir6.2/SUR1. However, these channels are not stimulated by pinacidil or cromakalim (48, 54). In contrast, Kir6.2/SUR2A K_{ATP} channels are not stimulated by diazoxide but are stimulated by pinacidil and cromakalim (16, 19). Similarly, Kir6.2/SUR2B K_{ATP} channels are stimulated by both pinacidil and cromakalim (16, 70). Since nucleotide binding and/or hydrolysis at both NBDs of SUR subunits are taken to be crucial for the specific binding and action of these potassium channel openers (19, 54, 70, 71) and for slowing down the off-rate of pinacidil (72), it is believed that the differences in sulphonylurea sensitivity between these channel subtypes may be caused partially by differences in nucleotide sensitivity of the different SUR isoforms (19).

Table II. The potassium channel openers that target the ATP-sensitive potassium channels

Agent	Pharmacologic class	Clinical use	As potential	Mechanism of action
Diazoxide		hypertensive crises		Opening of K_{ATP} channels causing K^+ efflux, hyperpolarization of the cell membrane and smooth muscle relaxation leading to vasodilation and drop in blood pressure
Minoxidil	potassium channel opener, vasodilator	hair growth stimulant, severe hypertension	antihypertensive and anti-asthmatic agents	
Nicorandil		angina pectoris		
Pinacidil		–		
Cromakalim		–		

The clinical use of potassium channel opening drugs has been limited due to the difficulties in developing tissue- and condition-selective K^+ channel-opening drugs (70). Different KCOs bind to different transmembrane polypeptide segments of the SUR. Within the 17 transmembrane polypeptide segments, the binding of pinacidil and cromakalim occurs at two domains within the helices numbered 16 and 17 (73), and in the cytoplasmic loop between segments 13 and 14 (74, 75). The binding site for diazoxide is not so well characterized. It is believed that diazoxide binding is nucleotide dependent (73). In SUR1, diazoxide binding has been mapped to bind between helices 8 to 11 and in the C-terminal region incorporating helix 17 and NBD2 (73).

One of KCOs functions is to activate $sarcK_{ATP}$ on vascular smooth muscle cells and cardiac myocytes, which leads to potassium ion efflux and membrane hyperpolarization. This in turn will reduce calcium influx and reduce the duration of the action potential, resulting in a negative inotropic effect in cardiomyocytes and vasodilatation of blood vessels (76, 77). First-generation drugs, including cromakalim and pinacidil, were aimed at treating hypertension; however, such medications failed to achieve clear benefits over angiotensin-converting enzyme inhibitors or calcium antagonists (78).

Selective KCO bimakalin did not show any anti-ischemic benefits in patients suffering from coronary artery disease during exercise-induced angina pectoris. However, the drug did cause a dose dependent vasodilatory activity (79). Studies, including one by Ueda *et al.* (80), have shown that KCO nicorandil administered intravenously can reduce the occurrence of ventricular fibrillation and QT dispersion in patients with acute myocardial infarction (AMI) undergoing successful coronary angioplasty. According to the Ueda study, this would prevent the occurrence of cardiac events following successful percutaneous transluminal coronary angioplasty for AMI patients. The administration of nicorandil to patients with stable angina in an angina (IONA) study (81) effectively reduced the rates of the combined primary endpoint of coronary heart disease mortality, myocardial infarction, or admission for chest pain, and the secondary endpoint of coronary heart disease mortality, myocardial infarction, or unstable angina and the rates of cardiovascular events.

Another K_{ATP} channel opener, levosimendan, which is a calcium sensitizer and inodilator, has been shown to significantly reduce pulmonary capillary wedge pressure in patients with severe low-output heart failure following cardiac surgery as well as in a case of peripartum cardiomyopathy (82). In controlled trials, levosimendan has been shown to decrease mortality rates in patients with severe low-output heart failure and in patients with left ventricular failure after AMI (83, 84).

CONCLUSIONS

In response to nucleotides and pharmaceutical agonists and antagonists, SUR allosterically regulate the K_{ATP} channel gating in a functional K_{ATP} channel complex $Kir6_4/SUR_4$. However, the transduction pathways for allosteric communication, which make the functional link between the pore forming Kir6 and the regulatory SUR subunits of K_{ATP} channels remain poorly understood.

To design novel and more effective drugs for the treatment of diabetes, high blood pressure and/or use as a cardioprotective agent in heart attack or cardiac surgery (therapeutic importance), the three-dimensional crystal structure of the functional K_{ATP} channel

needs to be determined. The crystal structure will clarify the allosteric communications between K_{ATP} channel subunits that induce the conformational changes and channel gating.

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