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PANCREATIC BETA CELL K^+ATP CHANNELS

Brijendra Kumar Srivastava, V Mohan

ABSTRACT

Sulphonylureas have been the backbone of the management of type 2 diabetes mellitus. As they interact with pancreatic beta cell ^{K}ATP channels in islets of Langerhans and stimulate insulin secretion, the ^{K}ATP channel has recently become a focus of attention of scientists. The beta cell ^{K}ATP channel is composed of Kir 6.2 / SUR1, in which the channel pore is formed by Kir 6.2. Although four ATP binding sites lie on the Kir 6.2, binding with one site is sufficient for channel closure. ATP inhibits the channel by interacting with Kir 6.2, while sulphonylurea blocks the channel activity by interaction with high affinity binding site on SUR1 and a low affinity site on Kir 6.2 suggesting a bivalent binding site for some sulphonylureas (e.g. glibenclamide). By cloning techniques, cDNA structure, gene structure and promoter sequences of SUR1 and Kir 6.2 have been studied in great detail and some diseases have been linked to mutations in the ^{K}ATP channels. Better understanding of ^{K}ATP channels could potentially help to develop newer therapeutic molecules that are safer, more effective and hopefully could prevent secondary failure to these agents in the future.

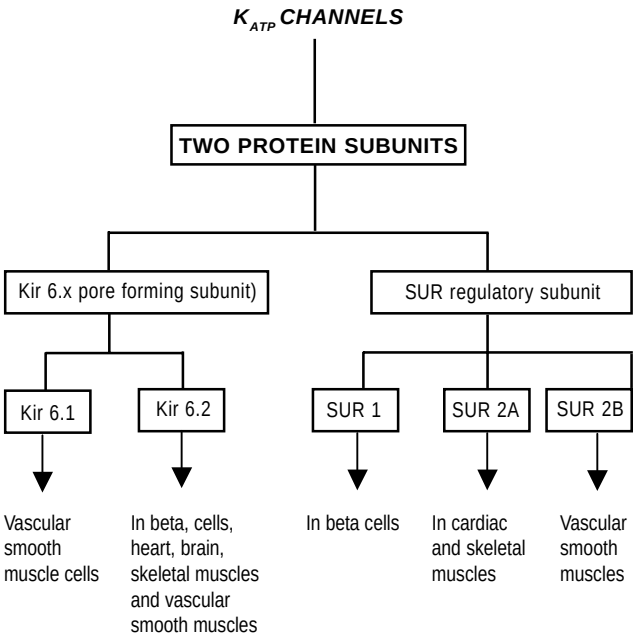
KEY WORDS: ^{K}ATP Channels; Transmembrane Domain 1: Transmembrane Domain 2: Sulphonylurea receptor 1: Kir 6.2.

Sulphonylureas (SU) have been the backbone of type 2 diabetes management for nearly five decades. It has been shown that they exert their hypoglycemic action by interacting with pancreatic beta cell ^{K}ATP channels. ^{K}ATP channels are also found in various other tissues like cardiac cells, neuronal cells, smooth muscle cells, kidneys and trachea. The sites of binding, the impact of the binding and structure and function of ^{K}ATP channel have been studied in great detail in recent years.

The ^{K}ATP channels couple cellular metabolism to electrical activity. The structure of this protein complex is unique among the ion channel families, as they are formed as a functional complex of two unrelated subunits, the Kir 6.X and a regulatory subunit, the sulphonylureas receptor SUR, which co-assembles with a 4:4 stoichiometry. Two different Kir 6.X genes have been described, Kir 6.1 and Kir 6.2. Two genes encoding sulphonylurea receptor, SUR 1 and SUR 2 have also been found. Splicing of SUR 2 results in two isoforms of SUR 2, SUR 2A, and SUR 2B (1-4).

The Kir 6.2 is strongly expressed in beta cells, heart, brain and skeletal muscles and forms the ^{K}ATP channel pore in these tissues, while it has been postulated that Kir 6.1 and Kir 6.2 both serve as the pore of the smooth muscle ^{K}ATP channels. SUR 1 is the predominant SUR subregulatory unit in beta cells and some type of neurons, while SUR 2A is found in cardiac and skeletal muscles and SUR 2B in smooth muscles (4) (Figure 1).

Figure 1: Different Types of ^{K}ATP Channels and their Arrangements.



Madras Diabetes Research Foundation, 35, Conran Smith Road, Gopalapuram, Chennai - 600 086, INDIA. Email:mvdsc@vsnl.in

Vascular smooth muscle cell ^{K}ATP channel (Kir 6.1 or Kir 6.2 / SUR 2B) are important in the control of contractile tone of vessels and may also help in control of blood pressure and blood flow. They can be opened pharmacologically by potassium channel opening drugs such as cromakalim and the vasodilator drug, diazoxide (5-8).

Under physiological conditions, cardiac and vascular smooth muscle cell ^{K}ATP channels remain closed. They open only in response to metabolic stress such as myocardial ischemia and/or hypoxia. Opening of these channels are dependent on the cytosolic concentrations of ATP. Opening of these ^{K}ATP channels leads to a series of events, the end results of which have been summarized in Figure 2 (9,10).

Sulphonylureas block the opening of these ^{K}ATPchannels and thus they may exert some beneficial and some harmful effects as well. The results are debatable and further experiments are needed to correlate with clinical outcomes.

Meglitinides are non-sulphonylurea benzamido derivatives. Meglitinides bind to ^{K}ATP channel at a different site than glibenclamide. Their binding with cardiac ^{K}ATP channel is debatable with one group of scientists documenting the binding, while others do not. However, as the drug has a plasma half-life of less than two hours, the chances of adverse cardiac side effects are minimal, if any (10-12).

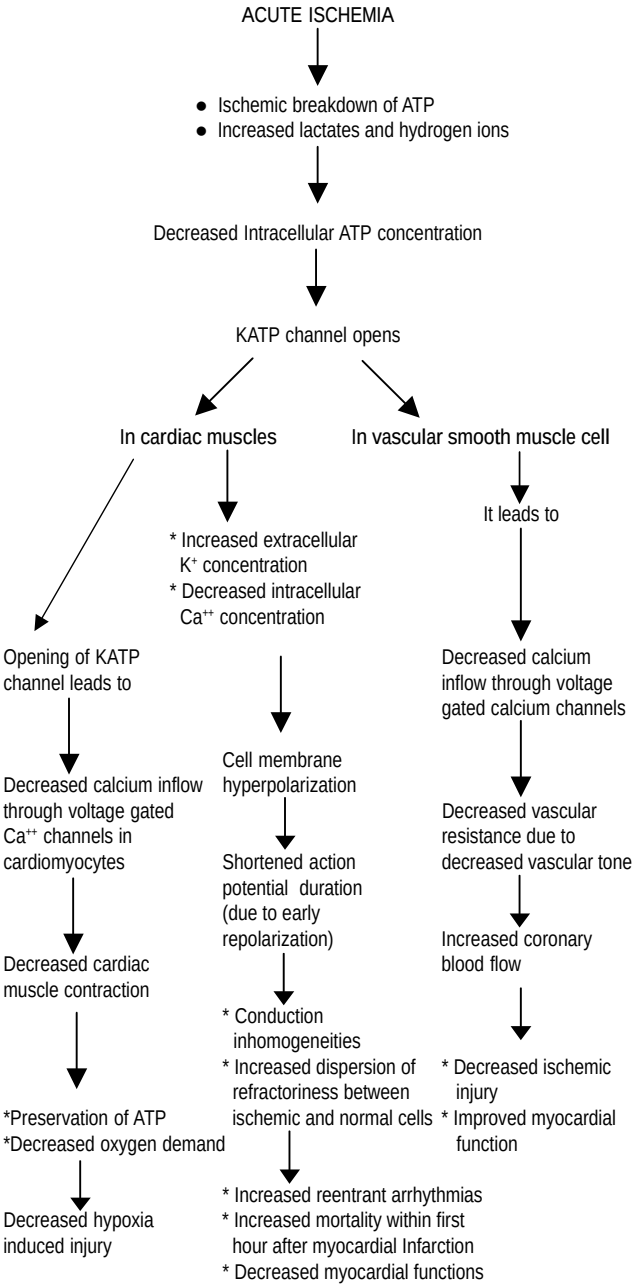
Mutations have been reported in Kir 6.2, SUR 1 and SUR 2. Three mutations that produce persisting hypoglycemia due to hyperinsulinemia, have been reported in Kir 6.2, which are as follows:

(i) A Leu→ Pro change, L147P, near the external side of M2 (leading to Persistent Hyperinsulinemic Hypoglycemia of Infancy).

(ii) A non-sense mutation in Kir 6.2 that truncates the protein after 12 amino acids, Y12X.

(iii) A, Kir 6.2 mutation, a Trp→ Arg change, W91R, near the external side of M_1 (13).

Figure 2: Effects Of Ischemia on Cardiac and Vascular Smooth Muscle Cell ^{K}ATP Channel.



Some of the SUR 2 cDNAs have been cloned like SUR 2A, SUR 2A, Δ17 and SUR 2A Δ17,18. Some SUR 1 mutations have also been reported which are:

i. Ag → a mutation, at a position –9 from the 3^{1} end of intron 32.

ii. Ag → a mutation in the last position of the exon 35.

iii. A deletion of codon 1388 resulting in a loss of phenylalanine residue ΔF1388

iv. Three mutations in the region of NBF1.

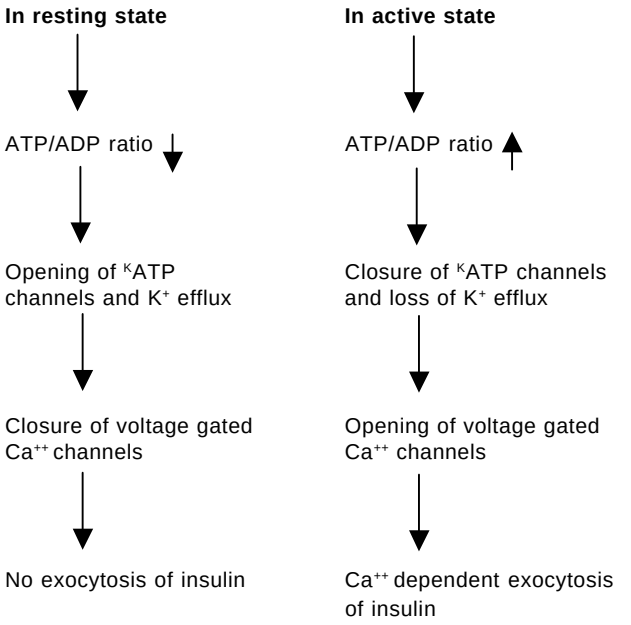
v. A missense mutation, G 1479 R, in the second NBF of SUR 1 (13,14).

A number of these mutations are associated with persistent hyperinsulinemic hypoglycemia of infancy, which is a rare metabolic disorder of neonates and infants characterized by inappropriate secretion of insulin despite severe hypoglycemia. Inappropriately raised plasma insulin levels to hypoglycemia and infusion of large amounts of glucose (more than 12 mg/kg/min) is required to maintain euglycemia in these new born infants as, otherwise irreversible brain damage can occur. Both familial as well as sporadic forms of the disease have been reported. It is an autosomal recessive disorder and a major susceptibility gene on the short arm of chromosome 11 at 11p14 – 15.1 has been mapped using multiple families. The reason for this disorder is the loss of normal pancreatic beta cell ^{K}ATP channel activity, thus effectively uncoupling the membrane electrical activity from metabolism and resulting in persistent insulin release, regardless of the blood glucose level, leading to severe hypoglycemia (13-15).

Sulphonylureas are the secretagogues, which stimulate insulin secretion from the existing viable pancreatic beta cells. They interact with pancreatic beta cell ^{K}ATP channels and stimulate insulin release. The ^{K}ATP channel is also known to play a major role in the glucose sensing apparatus of the pancreatic beta cells. Thus pancreatic beta cell ^{K}ATP channel needs to be studied in a great detail to understand its organization, structure and functions.

Insulin secretion is controlled by the membrane potential of the beta cells which depends on the activity of ^{K}ATP channels, in the plasma membrane. The ^{K}ATP channels close following an increase in the cytoplasmic ATP/ ADP ratio. This leads to membrane depolarization leading to opening of voltage gated calcium channels, elevation of cytoplasmic Ca^{++} concentration and stimulation of calcium dependent exocytosis of insulin containing granules (16). SU stimulate insulin secretion by binding to SUR 1 (sulphonylureas receptor 1) subunit of ^{K}ATP channels in the pancreatic beta cell plasma membrane and closing it. The rest of the events are same as above (Figure 3) (17).

Figure 3: Schematic Presentation of Insulin Secretion Mediated Through Pancreatic Beta Cell ^{K}ATP Channel.



The anionic group of these drugs favours the interaction of sulphonylurea binding with SUR1. Sulphonylureas owe their selectivity to SUR1 due to lipophilic substitution on their urea group (18). For example, glibenclamide interacts with both the high and low affinity binding sites on beta cells. The high affinity site lies on SUR1 while the low affinity site lies on Kir 6.2 subunits (19).

SUR 1 is the high affinity SU receptor which belongs to ABC transporter (ATP-Binding Cascade) family of proteins and has two nucleotide binding domains, NBD1 and NBD2 along with 17 putative transmembrane (TM) sequences arranged in three groups of 5,6 and 6 and a large number of possible protein kinase A, or C phosphorylation sites. The SUR1 is important in sensing nucleotide changes (13,20).

Diversity of SUR subunits underlies tissue specific pharmacology of ^{K}ATP channels. The pancreatic beta cell receptor, SUR 1, imparts high sensitivity to hypoglycemic SU's (glibenclamide) and low sensitivity to potassium channel openers (KCO's) (21,22).

Expression of either individual N or C terminal part of SUR1 alone gave no glibenclamide binding activity, confirming a bivalent structure of the glibenclamide binding site. In SUR1, in the native ^{K}ATP channel, close proximity of CL3 (cytosolic loop 3) and CL8 (cytosolic loop 8) leads to formation of the glibenclamide-binding site (23).

The serine residue at position 1237 of SUR1 which lies in the intracellular loop between TM15 and 16, appears to be critical for SU binding because its mutation to tyrosine abolished both tolbutamide block of ^{K}ATP currents as well as glibenclamide binding (24).

Kir 6.2 forms the pore of the ^{K}ATP channels. It belongs to a member of Kir inward rectifier potassium channel family (also known as Bir- beta cell inward rectifier). Immuno-fluorescence staining with antibody raised against Kir 6.2 revealed that Kir 6.2 protein is localized within the pancreatic islet and is not found in exocrine pancreas. The positive staining of Kir 6.2 appeared concentrated along the contour of each islet beta cells, suggesting that Kir 6.2 is at the plasma membrane of the beta cells (25). The N terminal end of Kir 6.2 subunit may be involved in coupling SUs binding to SUR1 leading to closure of the Kir 6.2 pore. Using novel trafficking based interaction assays, with immuno precipitation and current measurement; it has been shown that the first transmembrane segment and the N terminus of Kir 6.2 are involved in ^{K}ATP assembly and gating (26.27). The N terminus of Kir 6.2 is a determinant of the inter-burst kinetics of the beta cell ^{K}ATP channels and suggests that the two cytoplasmic domains of Kir 6.2 participate in ATP inhibitor gating through distinct mechanisms (28).

Cloning and redistribution of these subunits have studied the subunit stoichiometry of the pancreatic beta cell ATP sensitive K^{+} channel. It has been demonstrated that the activity of the ^{K}ATP channel is optimized when both the subunits are composed with a molar ratio of 1:1. The ^{K}ATP channel pore is lined by four Kir 6.2 subunits and each Kir 6.2 subunits requires one SUR1 subunit to generate a functional channel in a tetrameric or octameric structure. Thus the Kir 6.2 subunit forms the channel pore, whereas the SUR 1 is required for activation and regulation of the channel (29,30). The correct (SUR1 / Kir 6.2) stoichiometry of beta cell ^{K}ATP channels at the cell surface is tightly regulated by the presence of novel endoplasmic reticulum (ER) retention signals in SUR1 and Kir 6.2, so the incompletely assembled ^{K}ATP channels fails to exit the ER/Cis – Golgi compartments. In addition to these retrograde signals, the C-terminus of SUR 1 has an antegrade signal, composed in part of di-leucine motif and down stream phenylalanine, which is required for ^{K}ATP channels to exit the ER/Cis – Golgi compartments and transit to the cell surface (31,32).

The genes encoding human Kir 6.2 and SUR 1 have been cloned and they lie adjacent to one another on chromosome 11 p15.1. Transcriptional regulation of the two genes is important for correct tissue specific expression and to ensure that equal amounts of each subunit are produced (33,34).

The region specifying both the genes is contained within a segment that spans almost 90-kilo bases (kb) of DNA. SUR1 encodes the high affinity sulphonylureas receptor (1581 or 1582 amino acids). The intron less KCN gene encoding the 390 amino acid inward rectifier Kir 6.2 is 4900 base pairs 3^{1} of the end of SUR1 gene. The average exon size is 124 bp. It contains 39 exons (1581 amino acids) (14,33,34).

Increased intracellular ATP/ADP ratio closes the pancreatic beta cell ^{K}ATP channels, the receptor site for ATP is formed by Kir 6.2. Four identical non-cooperative ATP bindings sites are grouped within one ^{K}ATP channel complex, with occupation of one ATP binding site being sufficient to induce channel closure (35). Changes in ATP and ADP concentration gate the ^{K}ATP channel (36,37). The inhibitory action of ATP is modified by SUR1. ATP tonically inhibits ^{K}ATP channels, but ADP level in a fasting beta cell antagonizes this inhibition. The high affinity ATP binding site is the nuclear binding fold 1 of SUR1 (13,38-40).

Intracellular ADP enhances the inhibition of beta cell ^{K}ATP channel by SU's, because sulphonylurea by mediating through SUR1 prevents the stimulatory action of Mg^{++}ADP and unmasks the inhibitory effects of the nucleotide on Kir 6.2. The extent of inhibition is therefore determined by the combined blocking actions of the nucleotides and sulphonylureas. Mg^{++}ATP inhibited glibenclamide binding to sulphonylureas receptors by 25% by reducing the apparent number of glibenclamide binding sites, leaving the affinity unchanged (24,41).

The increased ^{K}ATP channel activity at low pH might have resulted from a mechanism involving an alteration of channel configuration. An increase in channel activity at low pH is one of the mechanisms underlying protein modulation of I ^{K}ATP in insulin secreting beta cells (42).

The effects of GTP binding proteins on ^{K}ATP channels have shown that G-Alpha II increases the activity of SUR1 – Kir 6.2 (pancreatic beta cell ^{K}ATP channel) by 200%, indicating that beta cell ^{K}ATP channels being modulated by G proteins (43). Potassium channel openers (KCO) bind to and act through sulphonylurea receptors. KCO binding to SUR requires ATP (as non hydrolysable ATP analogues do not support binding) (44,45).

SUR1 and Kir 6.2 mRNA levels are down regulated by approximately 40-50% in response to glucocorticoid treatment, as glucocorticoids most likely decrease the transcriptional activity of both SUR1 and Kir 6.2 genes (45).

High glucose concentration leads to markedly decreased (n-70%) Kir 6.2 mRNA and SUR1 mRNA levels in isolated rat pancreatic islets cells. This effect is reversible, because exposure to low glucose levels re-induces Kir 6.2 transcript levels (46).

Thus from the above discussion, it is clear that sulphonylurea receptor 1 is not only important in binding with SU drugs but also modulates several other functions in pancreatic beta cell ^{K}ATP channel, which are of immense interest as they ultimately affect the prime function of pancreatic beta cells i.e. insulin secretion.

Better understanding of ^{K}ATP channels could potentially help to develop newer therapeutic molecules. A novel drug would be that which should interact with a high affinity to pancreatic beta cell ^{K}ATP channel without having any binding with either cardiac or vascular smooth muscle cell ^{K}ATP channel, thus allowing them to function smoothly in response to hypoxia and ischemia. The binding at pancreatic beta cell should be readily reversible and the stimulation should not be a continuous one, so that the beta cell can get rest in between and in this way beta cell exhaustion could be avoided. At the same time, they should be effective in very small doses and maximal beneficial effects could be achieved with very low doses to avoid the untoward side effects of the drugs, if any.

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