REVIEW

The structure and function of the ATP-sensitive K⁺ channel in insulin-secreting pancreatic β-cells

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ABSTRACT

ATP-sensitive K⁺ channels (K_ATP channels) play important roles in many cellular functions by coupling cell metabolism to electrical activity. The K_ATP channels in pancreatic β-cells are thought to be critical in the regulation of glucose-induced and sulfonylurea-induced insulin secretion. Until recently, however, the molecular structure of the K_ATP channel was not known. Cloning members of the novel inwardly rectifying K⁺ channel subfamily Kir6·0 (Kir6·1 and Kir6·2) and the sulfonylurea receptors (SUR1 and SUR2) has clarified the molecular structure of K_ATP channels. The pancreatic β-cell K_ATP channel comprises two subunits: a Kir6·2 subunit and an SUR1 subunit. Molecular biological and molecular genetic studies have provided insights into the physiological and pathophysiological roles of the pancreatic β-cell K_ATP channel in insulin secretion.

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INTRODUCTION

Insulin secretion from pancreatic β-cells is essential in glucose homeostasis; it is regulated by many factors, including nutrients, hormones and neurotransmitters, among which glucose is physiologically the most important (Henquin 1994). The metabolism of glucose in pancreatic β-cells is the crucial step in glucose-induced insulin secretion. Pancreatic β-cells are electrically excitable cells (Dean & Matthews 1968), and glucose regulates insulin secretion by controlling K⁺ permeability, which determines membrane potential (Sehlin & Taljedal 1975, Henquin 1978). Thus, the K⁺ permeability of the β-cells is a critical determinant of glucose-induced insulin release. Before the identification of the ATP-sensitive K⁺ channels (K_ATP channels) in pancreatic β-cells, however, the molecule linking glucose metabolism and membrane potential was not known.

K_ATP channels were discovered originally in heart (Noma 1983), and were later found in many other tissues including pancreatic β-cells (Ashcroft et al. 1984, Cook & Hales 1984, Rorsman & Trube 1985), skeletal muscle (Spruce et al. 1985), smooth muscle (Studen et al. 1989), brain (Ashford et al. 1988), pituitary (Bernardi et al. 1988) and kidney (Hunter & Giebisch 1988). The activity of the K_ATP channels is controlled by intracellular ATP and ADP concentrations or the ATP/ADP ratio. An increase in the ATP/ADP ratio closes the K_ATP channels, while a decrease in the ratio opens them. K_ATP channels play a regulatory role in many cellular functions such as hormone secretion, excitability of neurons and muscles and cytoprotection in heart and brain ischemia, by linking the metabolic state of the cell to its membrane potential (Ashcroft et al. 1988, Terzic et al. 1995). The functional properties of K_ATP channels have been best characterized in pancreatic β-cells (Ashcroft & Rorsman 1989). Since the discovery of the K_ATP channels in β-cells, the model in which glucose-induced insulin secretion is dependent on the closure of the K_ATP channels has generally become accepted (Cook et al. 1988). In this model, the increase in ATP/ADP ratio due to the metabolism of glucose closes the K_ATP channels, depolarizing the β-cell membrane, leading to the opening of the voltage-dependent calcium channels, and allowing calcium influx. The rise in the intracellular calcium...
concentration ([Ca^{2+}]_i) in the β-cell then triggers insulin granule exocytosis. Accordingly, the K_{ATP} channels, as ATP and ADP sensors, are thought to be critical in the regulation of glucose-induced insulin secretion. In addition, sulfonylureas such as tolbutamide and glibenclamide, widely used in the treatment of non-insulin-dependent diabetes mellitus, stimulate insulin release by directly closing the K_{ATP} channels in pancreatic β-cells. This raised the possibility that the sulfonylurea receptor (SUR) itself is the K_{ATP} channel or that it is a regulatory protein associated closely with the K_{ATP} channel (Ashcroft 1988, Boyd 1988). The structure of K_{ATP} channels was revealed by the cloning of the SUR (Aguilar-Bryan et al. 1995) and members of a novel inwardly rectifying K+ channel (Kir6·0) subfamily (Inagaki et al. 1995a,b).

Since the K_{ATP} channels have inwardly rectifying K+ channel properties, it was assumed that the structure of K_{ATP} channels was similar to that of the other inwardly rectifying family members (Kir family) already known, which have two transmembrane segments (Jan & Jan 1994, Doupnik et al. 1995). Using GIRK1 (Kir3·1) cDNA as a probe, we identified a novel Kir member, uK_{ATP}-1 (now referred to as Kir6·1) from a rat islet cDNA library (Inagaki et al. 1995b). Since Kir6·1 shares only 40–45% amino acid identity with the known Kir members, it represented a new subfamily. Kir6·1 has two putative transmembrane segments with a K+ ion permeable domain, the H5 region. Northern blot analysis showed that Kir6·1 was ubiquitously expressed in tissues. However, Kir6·1 was not expressed in the various insulin-secreting cell lines such as MIN6, hamster insulinoma cell line (HIT) and rat insulinoma cell line RINm5F cells, all of which are known to have K_{ATP} channels, suggesting that there might be another inwardly rectifying K+ channel member specifically present in pancreatic β-cells. By using Kir6·1 cDNA as a probe, the new member, BIR (β-cell inward rectifier) was identified by screening a human genomic library and the MIN6 cDNA library (Inagaki et al. 1995a). Since BIR has 71% amino acid identity with Kir6·1, it is an isoform of Kir6·1, and, therefore, designated Kir6·2. Mouse Kir6·2 is a 390 amino acid protein. Northern blot analysis showed that Kir6·2 mRNA was expressed at very high levels in pancreatic islets and in the glucose-responsive insulin-secreting cell lines MIN6 and HIT, and is expressed at low to moderate levels in skeletal muscle, heart and brain, all of which are known to have K_{ATP} channels. However, expression of mouse Kir6·2 alone in COS-1 cells, HEK239 cells or Xenopus leavis oocytes elicited no significant K_{ATP} channel currents, indicating that Kir6·2 alone does not function as a K+ channel.

At almost the same time that Kir6·2 was cloned, the receptor for sulfonylurea (currently referred to as SUR1) was cloned by Aguilar-Bryan et al. (1998), Ashcroft & Gribble (1998) and Seino (1999).

### Molecular Structure of the Pancreatic β-Cell K_{ATP} Channel

Sulfonylureas are known to stimulate insulin secretion by directly closing the K_{ATP} channels in pancreatic β-cells. This raised the possibility that the sulfonylurea receptor (SUR) itself is the K_{ATP} channel or that it is a regulatory protein associated closely with the K_{ATP} channel (Ashcroft 1988, Boyd 1988). The structure of K_{ATP} channels was revealed by the cloning of the SUR (Aguilar-Bryan et al. 1995) and members of a novel inwardly rectifying K+ channel (Kir6·0) subfamily (Inagaki et al. 1995a,b).

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At almost the same time that Kir6·2 was cloned, the receptor for sulfonylurea (currently referred to as SUR1) was cloned by Aguilar-Bryan et al. (1998), Ashcroft & Gribble (1998) and Seino (1999).
They isolated a 140 kDa protein from HIT cell, which was photolabeled with a radio-iodinated derivative of glibenclamide, and a partial amino acid sequence of the 140 kDa protein was determined. Using the partial peptide sequences, SUR was cloned from HIT cell and rat insulinoma cell line (RINm5F) cDNA libraries. The hamster SUR1 is a 1582 amino acid protein and is expressed at high levels in pancreatic islets and pancreatic β-cell lines including MIN6, RINm5F and HIT (Inagaki et al. 1995). SUR1 mRNA is also expressed in small amounts in brain. SUR1 is structurally related to cystic fibrosis transmembrane conductance regulator (CFTR), multidrug resistant gene product (P-glycoprotein) and its related protein multidrug resistant associated protein, all of which belong to the ATP-binding cassette (ABC) transporter superfamily (Higgins 1992). SUR1 has multiple transmembrane segments and two nucleotide binding folds (NBF-1 and NBF-2) in the cytoplasmic side. There are Walker A and B motifs in both NBFs (Walker et al. 1982), which are important for the functional activity of many ABC proteins. SUR1 was originally proposed to have 13 transmembrane segments. Based on the sequence alignments with other ABC proteins, Tusnády et al. (1997) have proposed that SUR1 has 17 transmembrane segments. The expression of hamster or rat SUR1 in COSm6 cells exhibited a high binding affinity to glibenclamide with a $K_d$ of 10 nM or 2 nM for hamster or rat SUR1 respectively (Aguilar-Bryan et al. 1995). However, the expression of SUR1 alone or coexpression with Kir1·1a, Kir2·1 or Kir3·4 in Xenopus laevis oocytes generated neither ATP-sensitive nor glibenclamide-sensitive K$^+$ currents (Aguilar-Bryan et al. 1995).

To find if Kir6·2 functions as a channel by coupling to SUR1, we coexpressed Kir6·2 and SUR1 in COS-1 cells (Inagaki et al. 1995a). The coexpression of Kir6·2 and SUR1 elicited weakly inwardly rectifying K$^+$ channel currents with the single channel conductance of 76 pS at 140 mM K$^+$ on both sides of the membrane. The reconstituted channel activity was inhibited by ATP in a dose-dependent manner, with an IC$_{50}$ of $\sim$10 µM. The non-hydrolyzable ATP analog AMP-PNP also inhibited the channel activity. Glibenclamide strongly inhibited the channel activity reconstituted from Kir6·2 and SUR1. In contrast, 100 µM diazoxide, an opener of the β-cell K$_{ATP}$ channel, stimulated the activity. These properties of the channels reconstituted from SUR1 and Kir6·2 (SUR1/Kir6·2 channels) are similar to those of the K$_{ATP}$ channels in native pancreatic β-cells, indicating that the pancreatic β-cell K$_{ATP}$ channel comprises the two subunits, SUR1, a member of ABC transporter superfamily, and Kir6·2, a member of the inwardly rectifying K$^+$ channel family.

**MOLECULAR AND FUNCTIONAL DIVERSITY OF K$_{ATP}$ CHANNELS**

Since SUR1 was not expressed in cardiac and skeletal muscles in which K$_{ATP}$ channels are present, the presence of an isoform of SUR1 in these tissues was suggested. Screening brain and heart cDNA libraries identified an isoform of SUR1, SUR2 (currently referred to as SUR2A) (Inagaki et al. 1996). Rat SUR2A is a 1545 amino acid protein, having 68% identity with rat SUR1. However, the affinity of SUR2A for glibenclamide is $\sim$500 times lower than that of SUR1. SUR2A mRNA is expressed at high levels in heart, skeletal muscle and ovary and at moderate levels in pancreatic islets, brain, tongue, testis and adrenal gland. Coexpression of SUR2A and Kir6·2 in COS1 cells reconstituted weakly inwardly rectifying K$^+$ channel currents with single channel conductance identical to that of SUR1/Kir6·2 channels. Although ATP inhibits SUR2A/Kir6·2 channel activity in a dose-dependent manner, the SUR2A/Kir6·2 channel is less than a tenth as sensitive to ATP as the β-cell (SUR1/Kir6·2) K$_{ATP}$ channel.

**FIGURE 1.** A model for the structure of the pancreatic K$_{ATP}$ channel. The β-cell K$_{ATP}$ channel is probably a hetero-octamer composed of four Kir6·2 subunits and four SUR1 subunits.
Glibenclamide at 1 µM, the concentration sufficient to block β-cell K<sub>ATP</sub> channel currents, only slightly inhibited SUR2A/Kir6·2 channel activity. On the other hand, cardiac K<sub>ATP</sub> channel openers, cromakalim and pinacidil activated SUR2A/Kir6·2 channels. However, diazoxide at 100 µM, the concentration sufficient to activate β-cell K<sub>ATP</sub> channels, did not activate SUR2A/Kir6·2 channels. The electrophysiological and pharmacological properties of SUR2A/Kir6·2 channels, therefore, are similar to those of K<sub>ATP</sub> channels described in native heart and skeletal muscle (Inagaki et al. 1996).

In addition, Isomoto et al. (1996) have reported a variant of SUR2A, designated SUR2B. SUR2B is an alternatively spliced variant of SUR2A mRNA. As a result, the C-terminal 42 amino acid sequence of SUR2B is divergent from that of SUR2A, but similar to the C-terminus of SUR1. The coexpression of SUR2B and Kir6·2 elicits K<sub>ATP</sub> channel currents with properties similar to those of the K<sub>ATP</sub> channels in smooth muscle. Yamada et al. (1997) have shown that SUR2B and Kir6·1 reconstitute non-classical K<sub>ATP</sub> channels, that is, nucleotide diphosphate-dependent K<sup>+</sup> channels with properties similar to those of vascular smooth muscle K<sup>+</sup> channels. Another variant of SUR2 has also been identified (Chutkow et al. 1996). The tissue distribution of SUR1, SUR2A, Kir6·1 and Kir6·2 assessed by Northern blot analysis is summarized in Table 1.

Studies thus far suggest that differing combinations of SUR isoforms or their variants and Kir6·2 or Kir6·1 may account, in part, for the molecular and functional diversity of K<sub>ATP</sub> channels.

**NUCLEOTIDE BINDING IN THE PANCREATIC β-CELL K<sub>ATP</sub> CHANNEL AND CHANNEL REGULATION**

Physical association of the SUR1 subunit and Kir6·2 subunit has been shown by: co-photolabeling of SUR1 and Kir6·2 with <sup>125</sup>I-azidoglibenclamide in COSm6 cells transfected with SUR1 and Kir6·2; co-purification of the glycosylated SUR1 and Kir6·2 on wheat germ agglutinin agarose; Ni<sup>2+</sup>-agarose chromatography of histidine-tagged SUR1 plus Kir6·2 complexes (Clement et al. 1997); and co-immunoprecipitation of a mixture of the SUR1 subunit and Kir6·2 subunit in <i>in vitro</i>-translated proteins and from COS-7 cells transfected with both subunits (Lorenz et al. 1998). The SUR1 subunit and Kir6·2 subunit
have been shown to coassemble in an obligate 4:4 stoichiometry to form the β-cell K\textsubscript{ATP} channel (Clement \textit{et al.} 1997, Inagaki \textit{et al.} 1997, Shyng & Nichols 1997) (Fig. 1). The Kir6·2 subunits form the K\textsuperscript{+} ion permeation pathway. Since the SUR1 subunit has two NBFs, it is likely that the SUR1 subunit confers both ATP and ADP sensitivities. Photolabeling studies using 8-azido-[\(\alpha\textsuperscript{32P}\)]ATP and 8-azido-[\(\gamma\textsuperscript{32P}\)]ATP suggested that ATP strongly binds SUR1 in the absence of Mg\textsuperscript{2+} (Ueda \textit{et al.} 1997). Mutations of Walker A (K719R and K719M) in NBF-1 and Walker B (D854N) in NBF-1 of SUR1 severely impair Mg\textsuperscript{2+}-independent high-affinity ATP binding. MgADP antagonizes ATP binding at NBF-1 (IC\textsubscript{50}, \textasciitilde10 \mu M), and a mutation of NBF-2 (K1385M) reduces MgADP antagonism. Accordingly, it is thought that ATP binds to NBF-1 of SUR1 with high affinity, and MgADP, through binding to NBF-2, antagonizes the binding at NBF-1. These findings suggest that the SUR1 subunit might confer both ATP and MgADP-sensitivities in the β-cell K\textsubscript{ATP} channel. However, the electrophysiological studies indicated that although the mutations in the NBF-2 of SUR1 abolished MgADP activation of the β-cell K\textsubscript{ATP} channel (Nichols \textit{et al.} 1996, Gribble \textit{et al.} 1997, Shyng \textit{et al.} 1997), the mutations in the NBF-1 and/or NBF-2 of SUR1 did not alter ATP-sensitivity of the β-cell K\textsubscript{ATP} channel (Gribble \textit{et al.} 1997, Shyng \textit{et al.} 1997). In addition, Tucker \textit{et al.} (1997) have found that a truncation of the C-terminus of Kir6·2 (Kir6·2\texttilde C26) can produce K\textsubscript{ATP} channel currents in the absence of an SUR subunit. Coexpression of Kir6·2\texttilde C26 with the SUR1 subunit increases the ATP-sensitivity, however, and they found that both the N- and C-terminal regions of Kir6·2 are involved in the ATP-sensitivity (Tucker \textit{et al.} 1998). These electrophysiological findings suggest an alternative model, in which the Kir6·2 subunit confers the ATP-sensitivity primarily, while the SUR1 subunit confers MgADP activation through NBF-2 and enhances the sensitivity of the Kir6·2 channel to ATP (Shyng \textit{et al.} 1997, Tucker \textit{et al.} 1997). More recently, Gribble \textit{et al.} (1998) reported that MgATP activated K\textsubscript{ATP} channels reconstituted from SUR1 and a mutant Kir6·2 that impairs ATP inhibition (Kir6·2R50G), suggesting that MgATP

\textbf{FIGURE 3.} Possible models of β-cell K\textsubscript{ATP} channel dysfunction in PHHI (1) Physical uncoupling between SUR1 subunit and Kir6·2 subunit. Mutations of SUR1 or Kir6·2 subunit that disturb physical coupling between SUR1 subunit and Kir6·2 subunit could lock the K\textsubscript{ATP} channel in an inactive state. (2) Impaired MgADP activation. A point mutation in NBF2 of SUR1 (G1479R) has been shown to alter the sensitivity of K\textsubscript{ATP} to MgADP (Nichols \textit{et al.} 1996). This indicates that mutations in NBF-2 could abolish the channel activation by MgADP. (3) Impaired trafficking to the plasma membrane. Mutations in SUR1 could abolish the channel activity by impaired trafficking to the plasma membrane, as was found in CFTR mutations (Cheng \textit{et al.} 1990). (4) Impaired K\textsuperscript{+} ion permeation. Truncation of Kir6·2 subunit (Nestorowicz \textit{et al.} 1997) disrupts K\textsuperscript{+} ion permeable pore.
activates the β-cell K\textsubscript{ATP} channel through SUR1 under certain conditions. Thus, the relationship between nucleotide binding to the SUR1 or Kir6·2 subunit and gating of the K\textsubscript{ATP} channel remains to be clarified. Whether or not the SUR1 subunit has ATP-ase activity and how ATP hydrolysis is coupled to channel gating must also be determined.

**FAMILIAL PERSISTENT HYPERINSULINEMIC HYPOGLYCEMIA OF INFANCY**

Since the β-cell K\textsubscript{ATP} channel is a regulator of glucose-induced insulin secretion, genetic alterations of the K\textsubscript{ATP} channels could cause disorders of glucose homeostasis such as diabetes and hypoglycemia. Recent studies have shown that mutations of the SUR1 or Kir6·2 gene are associated with persistent hyperinsulinemic hypoglycemia of infancy (PHHI) (Aguilar-Bryan & Bryan 1996, Permutt et al. 1996, Thomas 1997), and abnormalities of the β-cell K\textsubscript{ATP} channel activity are responsible for the pathophysiology of PHHI (Kane et al. 1996).

PHHI is an autosomal recessive hereditary disease occurring in approximately 1/50 000 births in Western countries and in 1/2500 in some Arab communities (Aguilar-Bryan & Bryan 1996). The disease is characterized by severe hypoglycemia with inappropriate, excessive insulin secretion in neonates and infants. Genetic linkage analysis suggested that PHHI mapped to chromosome 11p14–11p15·1 (Glaser et al. 1994). The SUR1 and Kir6·2 genes were later found to be clustered at 11p15·1. The protein coding region of SUR1 and Kir6·2 is composed of 39 exons (Aguilar-Bryan et al. 1998) and a single exon (Inagaki et al. 1995a) respectively. A number of mutations in the SUR1 and Kir6·2 genes have been detected in PHHI patients (Fig. 2). Thomas et al. (1995) found two mutations (G1400D(23)X and 3990–9 guanine→adenine) within or preceding the NBF-2 region. The first mutation, G1400D(23)X, is a guanine to adenine mutation at the 3' end of exon 35, causing skipping of exon 35. This results in the substitution of glycine with aspartic acid at codon 1400 followed by the additional 23 extraneous amino acids. The seven affected children of nine families studied were homozygous for this guanine

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to adenine mutation. The second mutation (3990–9 guanine→adenine), found in another family, is a guanine to adenine homozygous mutation in the 3′ splice site 9 bp upstream from exon 33, which could generate three abnormal cryptic splice sites: a 7 bp addition, a 20 bp deletion or a 30 bp deletion in the NBF-2 region.

Three mutations of the NBF-1 region (G716V, 1672–20 adenine→guanine, and 2292–1 guanine→adenine) were also described (Thomas et al. 1996a). The affected children studied were homozygous for an amino acid substitution in Walker A motif of NBF-1 (G716V) and others were found to be compound heterozygous for two mutations in introns, 20 bp upstream from exon 12 and 1 bp upstream from exon 19 respectively. These mutations are thought to result in altered RNA processing in the NBF-1 region, suggesting the importance of NBF-1 in the activity of the K_ATP channel.

Other compound heterozygous mutations were found in Ashkenazi Jews. In this population, the combination of an in-frame deletion of phenylalanine at codon 1388 (Δ1388) and a 3′ splice mutation in intron 32 (3990–9 guanine→adenine) was responsible for 88% of the PHHI chromosomes (Nestorowicz et al. 1996).

A point mutation in exon 37 (G1479R), which is located proximal to the Walker B motif in NBF-2, was found in a PHHI patient by Nichols et al. (1996). The reconstituted K_ATP channel with this mutant SUR1 and Kir6·2 exhibited decreased sensitivity to MgADP and diazoxide. A guanine to adenine point mutation at the 3′ terminal nucleotide of exon 35 was detected in a PHHI patient of a Saudi Arabian family (Dunne et al. 1997).

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<th>Kir6·2G132S-Tg</th>
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<td>Stimulated</td>
<td>ND</td>
<td>Response (−)</td>
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<tr>
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<th>Kir6·2G132S-Tg</th>
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<tr>
<td><strong>Neonates</strong></td>
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<td>Fasting glucose levels</td>
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<td>Insulin response during IPGTT†</td>
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<td>β-cell number</td>
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<td>Frequency of apoptotic β-cells</td>
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*Resting membrane potential, basal [Ca²⁺], and basal insulin secretion were measured in the presence of 2·8 mM glucose. ND not determined. †IPGTT, intraperitoneal glucose loading test.
affected child was homozygous for this mutation. The site of the mutation is the same as that determined by Thomas et al. (1995). This mutation results in the substitution of arginine with glutamine at codon 1437 followed by the additional 23 extraneous amino acids (R1437Q(23)X). Co-transfection of the mutant SUR1 analogous to R1437Q(23)X and wild-type Kir6·2 did not elicit K\textsubscript{ATP} channel activity (Dunne et al. 1997).

Mutations of Kir6·2 also have been shown to cause PHHI. A leucine to proline mutation at codon 147 of the Kir6·2 gene (L147P) was detected in a severely affected PHHI child (Thomas et al. 1996b). The mutation is located in the conserved \(\alpha\)-helical second transmembrane (M2) of Kir6·2. A nonsense mutation at codon 12 of Kir6·2 (Y12X) found in a Saudi Arabian resulted in truncation of Kir6·2. Co-transfection of SUR1 and Kir6·2Y12X in COS-1 cells did not elicit any K\textsubscript{ATP} channel activity at all (Nestrowicz et al. 1997).

In PHHI patients, mutations of SUR1 or Kir6·2 induce loss of K\textsubscript{ATP} channel function (Kane et al. 1996). The mechanism of the K\textsubscript{ATP} channel dysfunction in PHHI could be physical uncoupling between the SUR1 subunit and the Kir6·2 subunit, impaired MgADP activation, impaired trafficking to the plasma membrane or impaired K\textsuperscript{+} ion permeation (Fig. 3). As a result, the pancreatic \(\beta\)-cells are continuously depolarized and allow continuous calcium influx, leading to hypoglycemia with unregulated insulin secretion. On the other hand, genetic studies suggest that neither the SUR1 nor the Kir6·2 gene is a major gene contributing to the development of diabetes (Iwasaki et al. 1996, Sakura et al. 1996, Inoue et al. 1997, Ohta et al. 1998).

STUDIES IN TRANSGENIC MICE

The K\textsuperscript{+} ion-permeable domain, H5, is highly conserved in K\textsuperscript{+} channels, and the motif Gly-Tyr (or Phe)-Gly is thought to be critical for K\textsuperscript{+} ion selectivity (Heginbotham et al. 1992, Jan & Jan 1994, Kerr & Sansom 1995). The mutant Kir6·2 with a substitution of the first residue of the Gly-Phe-Gly motif with serine, Kir6·2G132S, was shown to act as a dominant-negative inhibitor of K\textsubscript{ATP} channels, when coexpressed with wild-type SUR1 and Kir6·2. We generated transgenic mice expressing the mutant Kir6·2G132S specifically in pancreatic \(\beta\)-cells under the regulation of the human insulin promoter (Miki et al. 1997). Transgenic mice exhibited severe hypoglycemia in neonates, but the insulin levels remained relatively high, indicating that the phenotype resembles PHHI in the human. However, blood glucose levels in transgenic mice at 4 weeks became markedly elevated. The glucose-induced insulin secretion was markedly reduced in the transgenic mice, as assessed by an i.p. glucose tolerance test. K\textsubscript{ATP} channel conductance of the \(\beta\)-cells of transgenic mice was found to be significantly decreased. The resting membrane potential and basal [Ca\textsuperscript{2+}], of the \(\beta\)-cells of transgenic mice were significantly higher than those of control mice. Histological analysis revealed abnormal morphological configuration of the islet cells and a decrease in the number of \(\beta\)-cells in transgenic mice. Apoptotic \(\beta\)-cells were found at a relatively high frequency in the transgenic mice.

STUDIES IN KNOCKOUT MICE

The physiological role of K\textsubscript{ATP} channels in insulin secretion was further evaluated in mice lacking K\textsubscript{ATP} channels (K\textsubscript{ATP} channel knockout mice) (Miki et al. 1998). Since the Kir6·2 subunit forms the K\textsuperscript{+} ion-selective pore (Inagaki et al. 1996, Clement et al. 1997, Shyng & Nichols 1997), we assumed mice lacking K\textsubscript{ATP} channels could be generated by the disruption of Kir6·2 (Kir6·2\textsuperscript{−/−}). K\textsubscript{ATP} channel activity was absent in pancreatic \(\beta\)-cells of mice lacking Kir6·2 (Kir6·2\textsuperscript{−/−}), indicating that the Kir6·2 subunit is essential for functional K\textsubscript{ATP} channels. Kir6·2\textsuperscript{−/−} showed a transient hypoglycemia in neonates, similar to the transgenic mice described above. Although the blood glucose levels of Kir6·2\textsuperscript{−/−} in adults in the fed state were not significantly different from those of Kir6·2\textsuperscript{+/+}, the insulin response to glucose in vivo also was impaired in Kir6·2\textsuperscript{−/−}, as evaluated by i.p. glucose tolerance test. The studies of Kir6·2\textsuperscript{−/−} in vitro have revealed that the resting membrane potential and basal [Ca\textsuperscript{2+}], of pancreatic \(\beta\)-cells are determined primarily by the K\textsubscript{ATP} channels; the rise of [Ca\textsuperscript{2+}], in pancreatic \(\beta\)-cells requires closure of the K\textsubscript{ATP} channels; and both glucose-induced and sulfonylurea-induced insulin secretion require the rapid rise in [Ca\textsuperscript{2+}], caused by closure of the K\textsubscript{ATP} channels. Thus, glucose-induced and sulfonylurea-induced insulin secretion depends critically on the K\textsubscript{ATP} channel-dependent pathway (Fig. 4). These characteristics in mice expressing a dominant-negative Kir6·2 in pancreatic \(\beta\)-cells (Kir6·2G132S-Tg) and mice lacking Kir6·2 (Kir6·2\textsuperscript{−/−}) are summarized in Table 2.

CONCLUSIONS

Cloning the SURs and members of the inwardly rectifying K\textsuperscript{+} channel subfamily Kir6·0 has clarified
the molecular structure, functional diversity and regulation of $K_{ATP}$ channels. The pancreatic β-cell $K_{ATP}$ channel comprises four SUR1 subunits and four Kir6·2 subunits. The Kir6·2 subunits form the pore for $K^+$ permeation. ATP probably inhibits the channel by acting primarily on the Kir6·2 subunits; MgADP, sulfonylurea, and diazoxide act primarily on the SUR1 subunits. Studies of dominant-negative Kir6·2 transgenic mice and Kir6·2 knockout mice have shown directly that the $K_{ATP}$ channel in pancreatic β-cells is an important molecule for both glucose-induced and sulfonylurea-induced insulin secretion. However, there are many issues to be resolved. How is nucleotide binding to SUR1 and Kir6·2 coupled to channel gating? Does the SUR subunit have ATP-ase activity? Does phosphorylation of SUR1 or Kir6·2 affect the channel activity? Is there any third factor that modulates $K_{ATP}$ channel activity by interacting with SUR1 or Kir6·2 subunit? What is the molecular basis of the mitochondrial $K_{ATP}$ channel in pancreatic β-cells? Studies addressing these issues are currently under way in many laboratories.

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