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ATP-sensitive K^+ Channels: Structure, Function, and Physiological Roles

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INTRODUCTION

ATP-sensitive K^+ (K) channels were discovered originally in heart, and were later found in many other tissues including pancreatic β-cells. K_{ATP} channels, acting as ATP and ADP sensors, play important roles in many cellular functions by coupling cell metabolism to membrane potential. Cloning of members of the inwardly rectifying K^+ channel subfamily Kir6.0 (Kir6.1 and Kir6.2) and sulfonylurea receptors (SUR1 and SUR2) has clarified the structure of K channels and led to a new paradigm of channel/receptor assemblies (Ashcroft & Gribble, 1988; Aguilar-Bryan & Bryan, 1999; Seino, 1999). The pancreatic β-cell K_{ATP} channel comprises Kir6.2 subunits and SUR1 subunits with 4 : 4 stoichiometry. While the Kir6.2 subunits form the K^+ ion permeable pore, and confer primarily inhibition by ATP, the SUR1 subunits mediate the stimulatory effects of MgADP, and are the primary target for the pharmacological agents such as sulfonylureas and K^+ channel openers.

The inwardly rectifying K^+ channel subfamily member Kir6.2

cDNA encoding Kir6.1, a member of the Kir family (Kir6.0 subfamily) with two transmembrane segments was cloned from a cDNA library constructed from rat islet mRNA (Inagaki et al, 1995c). Although Kir6.1 is expressed widely in tissues including pancreatic islets, heart, skeletal muscle, and brain, it is not expressed in any of the insulin-secreting cell lines examined. Human Kir6.1, KCNJ8, was mapped to chromosome 12p11.23 using fluorescence *in situ* hybridization (Inagaki et al, 1995b); the gene contains 3 exons spanning about 10 kb. Using Kir6.1 cDNA as a probe, Kir6.2, an isoform of Kir6.1, was subsequently cloned (Inagaki et al, 1995a). Mouse Kir6.2 is a protein of 390 amino acids that shares 71% amino acid identity (82% similarity) with rat Kir6.1. Although the Gly-Tyr-Gly motif in the H5 region, which is thought to be critical for K^+ ion selectivity, is highly conserved in inwardly rectifying K^+ channels, the amino acid sequence of this motif in both Kir6.1 and Kir6.2 is Gly-Phe-Gly, suggesting that this motif may be unique in the Kir6.0 subfamily. Two potential protein kinase A-dependent phosphorylation sites (Thr-224 and Ser-372) in Kir6.2 are conserved in all species in which it has been identified to date. Kir6.2 mRNA is expressed at high levels in pancreatic islets, the glucose-responsive insulin-secreting cell lines MIN6 (mouse) and HIT-T15 (hamster), and is expressed also in heart, skeletal muscle, and brain. *In situ* hybridization and immunohistochemistry reveals that Kir6.2 is present in the insulin-secreting β-cells, glucagon-secreting α-cells, somatostatin-secreting γ-cells, and pancreatic polypeptide (PP)-secreting cells in pancreatic islets (Suzuki et al, 1997). The Kir6.2 gene has no intron in the protein-coding region. Human Kir6.2, KCNJ11, was mapped to 11p15.1 by fluorescence *in situ* hybridization (Inagaki et al, 1995a).

The sulfonylurea receptor

The sulfonylurea receptor (SUR1) that shows high affinity for glibenclamide was cloned from HIT and RINm5F cDNA libraries (Aguilar-Bryan et al, 1995). Hamster SUR1 and rat SUR1 have been reported to be a 1581 and a 1582 amino acid protein, respectively. SUR1 has multiple putative transmembrane segments and two nucleotide binding folds (or domains) (NBFs or NBDs) in the cytoplasmic side.

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As with cystic fibrosis transmembrane conductance regulator (CFTR), P-glycoprotein (P-gp), and multidrug resistance associated protein (MRP), SUR1 is a member of the ABC superfamily (Aguilar-Bryan et al, 1995). Each NBF contains the Walker A and B motifs and the SGGQ ABC signature, which are thought to be important in nucleotide regulation of the functional activity of ABC proteins (Higgins, 1992). Although SUR1 was originally proposed to have 13 transmembrane segments, it has recently been proposed to represent a 17 transmembrane segment model, based on sequence alignments of SUR1 and members of the MRP gene subfamily (Thsnády et al, 1997). In this model, in addition to two transmembrane domains (TMD1 and TMD2), each of which consists of 6 transmembrane segments, SUR1 has another transmembrane domain (TMD0) consisting of 5 transmembrane segments in the N-terminal region. There are three potential protein kinase A-dependent phosphorylation sites in rat SUR1 (Thr-278, Ser-1448, Ser-1500), four in hamster (Thr-278, Ser-950, Ser-1447, Ser-1501), and four in human (Thr-949, Ser-1446, Ser-1500, and Ser-1571). cDNA encoding an isoform of SUR1, SUR2 (now called SUR2A) was subsequently cloned (Inagaki et al, 1996). Rat SUR2 is a protein of 1545 amino acids sharing 68% amino acid identity with hamster SUR1 and also similar membrane topology. There are three potential N-linked glycosylation sites in rat SUR2: one in the extracellular N-terminus (Asn-9) and two in the extracellular region between the 6th and 7th, (Asn-330 and Asn-331) transmem brane segment. There are two potential protein kinase A-dependent phosphorylation sites: one (T-632) in the 11th and 12th, and one (S-1464) in the intracellular C-terminal region in rat SUR2A. One variant, SUR2B, differs from SUR2A by 42 amino acids in the C-terminus, but is similar in the C-terminus to SUR1 (Isomoto et al, 1996). While SUR2A is expressed predominantly in heart and skeletal muscle (Inagaki et al, 1996), SUR2B is expressed ubiquitously (Isomoto et al, 1996). In addition to these main transcripts, other alternative spliced mRNAs also have been identified (SUR2AΔ14: a deletion of exon 14 (Chutkow et al, 1996), SUR2AΔ17 and Δ17, 18: a deletion of exon 17 or exon 17 plus 18 (Chutkow et al, 1999)). The human SUR1 gene, spanning 39 exons over 100 kb of DNA. The human SUR2 gene, spanning 38 exons over 100 kb (Aguilar-Bryan & Bryan, 1999), localizes on the short arm of chromosome 12 at 12p11.12.

Reconstitution of K_{ATP} channels

Coexpression of Kir6.2 and SUR1 in COS-1 cells reconstitutes weakly inwardly rectifying channel K^+ currents (Inagaki et al, 1995a). The reconstituted K^+ channel (Kir6.2/SUR1 channel) currents are inhibited by ATP with half- maximal inhibition (Ki) at $\sim$10 μM in the presence of Mg^{2+}. The reconstituted currents are also inhibited by adenyl-5'-yl imidodiphosphate (AMP-PNP), a non-hydrolysable ATP analog. Glibenclamide blocks the reconstituted K^+ channel currents, while diazoxide stimulates them. Thus, the properties of K^+ channel currents reconstituted from Kir6.2 and SUR1 are similar to those of the K_{ATP} channel currents in native pancreatic β-cells, indicating that Kir6.2 and SUR1 couple to form the functionall pancreatic β-cell type K_{ATP} channels.

Differing combinations of the Kir6.2 or Kir6.1 and the SUR1 or SUR2 variants comprise K_{ATP} channels with distinct nucleotide and pharmacological sensitivities: Kir6.2 and SUR2A constitute cardiac type and, probably, skeletal muscle type K_{ATP} channels (Inagaki et al, 1996); Kir6.1 and SUR2B reconstitute ATP-insensitive, nucleotide diphosphate-activated, and glibenclamide-sensitive K^+ channel currents resembling the nucleotide diphosphate-dependent (K_{NDP}) K^+ channels in vascular smooth muscle (Yamada et al, 1997).

Regulation of the β-cell K_{ATP} channel activity by PKA-mediated phosphorylation

The molecular mechanism by which hormones and neurotransmitters modulate K^+ channels via protein kinase A (PKA) is poorly understood. We identified the sites responsible for PKA phosphorylation in the C-terminus of Kir6.2 (S-372) and SUR1 (S-1571). Kir6.2 can be phosphorylated at its PKA phosphorylation site in intact cells after G-protein (Gs) coupled receptor or direct PKA stimulation. While the phosphorylation of Kir6.2 increases channel activity, the phosphorylation of SUR1 contributes to the basal channel properties by decreasing burst duration, interburst interval and open probability, and also increases the number of functional channels at the cell surface (Béguin et al, 1999). These data demonstrate direct phosphorylation by PKA of the pancreatic β-cell K_{ATP} channel, and may explain the mechanism by which Gs protein coupled receptors stimulate channel activity. Importantly, the data also describe a model

of heteromultimeric ion channels in which there are functionally distinct roles of the phosphorylation of the different subunits.

Physiological roles of the β-cell K_{ATP} channels

Recent studies have shown that mutations of the SUR1 or Kir6.2 gene can cause persistent hyperinsulinemic hypoglycemia of infancy (PHHI) (Seino, 1999). To clarify the physiological roles of K_{ATP} channels directly, we generated two types of genetically engineered mice: mice expressing a dominant-negative form of Kir6.2 specifically in pancreatic β-cells (Tg mice) (Miki et al, 1997) and mice lacking Kir6.2 (KO mice) (Miki et al, 1998). The K^+ ion-permeable domain, H5, is highly conserved in K^+ channels, and the motif Gly-Tyr (or Phe)-Gly is thought to be critical for K^+ ion selectivity. 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_{ATP} channels, as assessed by $^{86}Rb^+$ efflux from COS-1 cells transfected with Kir6.2 and Kir6.2G132S at various ratios together with SUR1. Tg mice expressing the mutant Kir6.2G132S specifically in pancreatic β-cells under the regulation of the human insulin promoter were then generated. The transgenic mice exhibited severe hypoglycemia in neonates, but the insulin levels remained relatively high, indicating that the phenotype resembles PHHI in human. However, blood glucose levels in the Tg mice at 4 weeks became markedly elevated and increased further with age. The glucose-induced insulin secretion was markedly reduced in the Tg mice, as assessed by intraperitoneal glucose tolerance test. K_{ATP} channel conductance of the β-cells of transgenic mice was found to be significantly decreased. The resting membrane potential and basal intracellular calcium concentrations ($[Ca^{2+}]_i$) of the β-cells in transgenic mice were significantly higher than those of control mice. Histological analysis revealed that apoptotic β-cell death is detected frequently by TUNEL method in the Tg mice. These results suggest that the loss of β-cells in the Tg mice is due to acceleration of apoptosis.

The physiological roles of K_{ATP} channels were further evaluated in mice lacking K channels (knockout mice). Since the Kir6.2 subunit forms the K^+ ion-selective pore, we assumed that mice lacking K channels could be generated by the disruption of the Kir6.2 gene ($Kir6.2^{-/-}$). In fact, K channel activity was completely absent in pancreatic β-cells of mice lacking Kir6.2 ($Kir6.2^{-/-}$), as assessed by whole-cell and single channel recordings of pancreatic β-cells. This indicates that Kir6.2 subunit is essential for functional K^+ channels. $Kir6.2^{-/-}$ mice showed a transient hypoglycemia in neonates, similar to the transgenic mice. The resting membrane potential and basal intracellular calcium concentrations ($[Ca^{2+}]_i$) of the β-cells in $Kir6.2^{-/-}$ were significantly higher than those of control mice, but they do not respond at all to either high glucose or the sulfonylurea tolbutamide stimulation. Perifusion experiments of pancreatic islets show that neither glucose at high concentrations nor tolbutamide elicited significant insulin secretion in K_{ATP} channel-deficient mice, indicating that both glucose-induced and tolbutamide-induced insulin secretion depend critically upon the K_{ATP} channel activity. Histological examination shows that glucagon-positive β-cells, which are present primarily in the periphery in islets of normal mice, appear also in the central region in islets of $Kir6.2^{-/-}$. This finding is similar to that in the Kir6.2G132S Tg mice. The studies of the Tg mice and KO mice indicate clearly that the β-cell K_{ATP} channel plays a critical role in glucose-induced and sulfonylurea-induced insulin secretion and suggest that the K_{ATP} channel in skeletal muscle may be involved in insulin-induced glucose transport. K_{ATP} channels may also play a role in differentiation and proliferation of pancreatic islet cells.

CONCLUSIONS

Cloning the sulfonylurea receptors and members of the inwardly rectifying K^+ channel subfamily Kir6.0 has clarified the molecular structure, functional diversity, and regulation of K_{ATP} channels. The pancreatic β-cell K channel comprises four SUR1 subunits and four Kir6.2 subunits. Studies of these two mouse lines with impaired KATP channel function show clearly that the KATP channels in pancreatic β-cells are critical in both glucose-induced and sulfonylurea-induced insulin secretion. They also suggest the important roles of the KATP channels in β-cell survival and differentiation of islet endocrine cells.

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