Of Mice and Men: \( K_{\text{ATP}} \) Channels and Insulin Secretion

LYDIA AGUILAR-BRYAN,* JOSEPH BRYAN,† AND MITSUHIRO NAKAZAKI‡‡

*Departments of Medicine and †Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas 77030; ‡‡The First Department of Internal Medicine, Faculty of Medicine, Kagoshima University, Kagoshima. 890-8520. Japan

ABSTRACT

\( K_{\text{ATP}} \) channels are a unique, small family of potassium (K\(^+\))-selective ion channels assembled from four inward rectifier pore-forming subunits, \( K_{\text{ir}} \), paired with four sulfonylurea receptors (SURs), members of the adenosine triphosphate (ATP)-binding cassette superfamily. The activity of these channels can be regulated by metabolically driven changes in the ratio of adenosine diphosphate (ADP) to ATP, providing a means to couple membrane electrical activity with metabolism. In pancreatic \( \beta \) cells in the islets of Langerhans, \( K_{\text{ATP}} \) channels are part of an ionic mechanism that couples glucose metabolism to insulin secretion. This chapter 1) briefly describes the properties of \( K_{\text{ATP}} \) channels; 2) discusses data on a genetically recessive form of persistent hyperinsulinemic hypoglycemia of infancy (PHHI), caused by loss of \( \beta \)-cell \( K_{\text{ATP}} \) channel activity; and 3) compares the severe impairment of glucose homeostasis that characterizes the human phenotype with the near-normal phenotype observed in \( K_{\text{ATP}} \) channel null mice.

I. Introduction

A wide range of factors or processes, often classified arbitrarily by their effects on insulin secretion vs. insulin action, are known to affect glucose homeostasis and produce either hypoglycemia or hyperglycemia. The hyperglycemia characteristic of type II or non-insulin dependent diabetes is associated with a decreased sensitivity of different tissues to the normal glucose-lowering action of insulin (insulin resistance) and a failure of pancreatic \( \beta \) cells to produce sufficient insulin. The hyperglycemia present in type I or insulin-dependent diabetes is the result of reduced or absent insulin secretion secondary to the immunologic destruction of insulin-secreting \( \beta \) cells in the islets of Langerhans within the pancreas. While the molecular mechanism(s) of insulin resistance and \( \beta \)-cell destruction are not completely understood, an enormous amount of work has been done in these areas for more than two decades.

Profound hypoglycemic states in adults, although rare, have been associated with hyperinsulinism resulting from insulinomas whose insulin secretion is inappropriately regulated. However, the factors leading to uncontrolled insulin secre-
tion in nontumor states are less well understood. During the last half-dozen years, insight into the genetic basis of congenital hyperinsulinism, or persistent hyperinsulinemic hypoglycemia of infancy (HI or PHHI), has begun to provide deeper understanding of the normal regulation of insulin secretion and the types of metabolic defects that can produce this disorder. In overall outline, this work has largely confirmed the early ionic steps in a basic regulatory model developed by a number of workers over the past 20 years. In this model, the metabolism of glucose is coupled to the membrane electrical activity of β cells, which controls the influx of calcium ions that initiate and potentiate insulin exocytosis. In the early steps of this model, glucose metabolism in β cells, initiated by glucokinase (GCK), decreases the adenosine diphosphate/adenosine triphosphate (ADP/ATP) ratio. This, in turn, reduces the activity of ATP-sensitive K⁺ channels (K_{ATP} channels). In low-glucose conditions, K_{ATP} channels act to hyperpolarize the β-cell membrane. A decrease in their activity results in membrane depolarization and initiation of Ca^{2+}-dependent action potentials. These action potentials involve both voltage-sensitive “L-type” Ca^{2+} channels, whose openings further depolarize the β-cell membrane, and other poorly characterized repolarizing currents. The increased Ca^{2+} influx through the L-type Ca^{2+} channels increases [Ca^{2+}]. This stimulates insulin exocytosis in concert with other second messengers, including cyclic nucleotides, the hydrolytic products of phospholipases (A2, C, and D), and adenine and guanine nucleotides. Thus, Ca^{2+} facilitates exocytosis and K_{ATP} channels regulate glucose-stimulated insulin secretion by controlling Ca^{2+} channel activity.

II. Persistent Hyperinsulinemic Hypoglycemia of Infancy

PHHI, also referred to as familial hyperinsulinism or pancreatic nesidioblastosis (Online Mendelian Inheritance in Man: OMIM 256450), is a metabolic disorder of neonates identified by inappropriate secretion of insulin in the presence of hypoglycemia (for recent reviews, see Aguilar-Bryan and Bryan, 1996,1999; Permutt et al., 1996; Glaser et al., 1999; Bryan and Aguilar-Bryan, 2000; Glaser, 2000; and Shepherd et al., 2000). Plasma insulin is often elevated, the key feature being an inappropriately high level for the degree of hypoglycemia. Infusion of large amounts of glucose, more than 12 mg/kg/min, is required to maintain euglycemia in newborns. In severe cases, patients are unresponsive to K_{ATP} channel openers (e.g., diazoxide) or to treatment with somatostatin derivatives (e.g., octreotide). Partial or subtotal pancreatectomy is done to relieve the hypoglycemia (Aynsley-Green et al., 2000).

The diagnosis of hyperinsulinism — considered here as high insulin levels, low blood ketone levels, and low free fatty acid levels at the time of hypoglycemia — has been shown to result from three genetic defects. At the suggestion of Franz
Matschinsky, we (Aguilar-Bryan and Bryan, 1999) have designated these subclasses of hyperinsulinism as “HI-,” followed by the protein affected (e.g., HI-GCK, HI-SUR). GCK, the type IV hexokinase found in β cells, converts glucose to glucose-6-phosphate and is generally considered to be the β-cell “glucose sensor” (Matschinsky, 1996). GCK mutations have been identified as one cause of maturity onset diabetes of the young (MODY 2). In these cases, an increase in the $K_m$ of the enzyme is correlated with an increase in the glucose concentration needed for insulin release. Glaser et al. (1998) have described a “gain-of-function” mutation in GCK that lowers the $K_m$ and results in hypoglycemia. This mutation, val → met, V408M, causes a rare, mild, dominant form of PHHI, HI-GCK (OMIM 602485), that responds clinically to diazoxide. No homozygotes have been identified, suggesting two copies of the allele may be lethal. The result is consistent with the basic model, as the mutant enzyme should make β cells more “efficient” by increasing the rate of glucose metabolism at lower glucose concentrations, thus reducing the glucose “setpoint.”

Zammarchi et al. (1996) and Weinzimer et al. (1997) have described a different mild, dominant form of hyperinsulinism in which the affected individuals also present moderate asymptomatic hyperammonemia. Several mutations in the glutamate dehydrogenase (GLUD1) gene have been identified in isolated and familial cases, in patients that have normal weight at birth and a good response to diazoxide. This form of hyperinsulinism, HI-GLUD1 (OMIM 138130), appears to be the result of an increase in GLUD1 activity that produces a subsequent increase in α-ketoglutarate production and excessive ATP. Enzyme studies indicate that the glutamate dehydrogenase in two unrelated patients with this disorder was less sensitive to GTP, a negative allosteric effector as a result of mutations in the C-terminus of the enzyme (Stanley et al., 1997).

This review is focused on a third group of mutations, discovered prior to those described above, which are found in the two subunits that make up β cell KATP channels (Inagaki et al., 1995a). These mutations cause PHHI, termed HI-SUR1 (OMIM 600509) or HI-Kr6.2 (OMIM 600937), depending on the affected subunit. This subclass of HI usually is considered to be recessive and can be either mild or severe, conditions usually distinguished by the clinical symptoms and the lack of response to diazoxide. Mutations in these two genes are inherited in a Mendelian way but loss of the maternal allele(s), in parallel with a heterozygous germline mutation in the paternal SUR1 allele, also has been described (Ryan et al., 1998; Verkarre et al., 1998). Mild forms have been associated with missense mutations in the regulatory subunit (SUR1), which produce channels that can be inhibited by ATP, are poorly stimulated by the addition of MgADP, but remain sensitive to diazoxide (Nichols et al., 1996; Shyng et al., 1998). Severe forms of the disease have been associated with splice site and nonsense mutations that truncate the regulatory subunit (Thomas et al., 1995; Dunne et al., 1997) and block
expression of channels at the cell surface by inhibiting normal trafficking to the plasma membrane (Sharma et al., 1999). Similarly, a severely truncated form of K_{IR}6.2 causes PHHI (Nestorowicz et al., 1997). These results were consistent with the basic model, which suggested that secondary to loss of K_{ATP} channels, PHHI β cells should display a predictable electrical phenotype characterized by persistent depolarization, the presence of Ca^{2+}-dependent action potentials, and elevated [Ca^{2+}]. Analysis of human PHHI β cells has largely confirmed this expectation (Dunne et al., 1997; Otonkoski et al., 1999), while observations on insulin release from cultured PHHI β cells (Kaiser et al., 1990) and the increased insulin levels in PHHI patients suggested that elevated Ca^{2+} increased insulin exocytosis.

We have reviewed all aspects of this area over the past several years (Aguilar-Bryan and Bryan, 1996, 1999; Aguilar-Bryan et al., 1998; Bryan and Aguilar-Bryan, 1997, 1999, 2000; Babenko et al., 1998). Our intention in this chapter is to minimize overlap with these previous publications. Rather, we review briefly recent studies aimed at understanding how particular K_{ATP} channel mutations cause PHHI. We want to draw attention to the rather remarkable physiologic differences between human neonates missing K_{ATP} channel activity and K_{ATP} channel knockout mice. We have tried to provide the reader with the minimal background in K_{ATP} channels needed to follow the story and have made frequent reference to earlier reviews where additional detail can be found.

III. K_{ATP} Channels Are Assembled from SURs and K_{IRS}

K_{ATP} channels initially were identified in cardiac myocytes (Noma, 1983; Trube and Hescheler, 1983, 1984) and pancreatic β cells (Cook and Hales, 1984). Their biophysical and pharmacologic properties have been studied intensively (Nelson and Quayle, 1995; Dukes and Philipson, 1996; Tucker et al., 1998). Our understanding of these channels increased markedly with the cloning of both their pore (K_{IR}6.x) (Inagaki et al., 1995a, 1995b) and regulatory (SUR) (Aguilar-Bryan et al., 1995) subunits and with the reconstitution of the β cell (Inagaki et al., 1995a), cardiac (Inagaki et al., 1996; Babenko et al., 1998b), and smooth muscle (Isomoto et al., 1996; Yamada et al., 1997) type channels. The cloning and reconstitution studies established that K_{ATP} channels are a unique combination of two quite dissimilar subunits: 1) a large, regulatory subunit with homology to members of the transport ATPase superfamily, particularly the multidrug resistance (MDR) and multidrug resistance-associated proteins (MRP), and 2) a small, potassium ion channel subunit that forms the channel pore. While other transport ATPases couple ATP hydrolysis to movement of molecules through a membrane against their concentration gradient, SURs, the regulatory subunits of K_{ATP} channels, control the flux of potassium ions through a pore driven by a concentration gradient or, more precisely, by the difference between the Nernst potential result-
ing from a \( K^+ \) gradient and the membrane potential. Control is exerted in response to changes in the concentrations of cytosolic ADP/ATP that result from ATP synthesis and hydrolysis within the cell and presumably on the regulatory subunit itself. Thus, ATP hydrolysis is thought to occur as an integral part of the regulation of K\(_{\text{ATP}}\) channels, although it does not power \( K^+ \) movement.

The regulatory subunits are termed SURs because they bind hypoglycemic sulfonylureas. The \( \beta \)-cell receptor, for example, binds glibenclamide with high affinity (\( K_D \) in the low nM range). The SURs consist of a small family, specified by the Sur1 and Sur2 genes that encode the high-affinity SUR1 and low-affinity SUR2A and SUR2B receptors, respectively. The pore subunits are termed K\(_{\text{IRs}}\), or potassium inward rectifiers (see Doupnik et al., 1995, and Nichols and Lopatin, 1997, for reviews). Rectifiers are devices for converting alternating currents into direct currents — in short, devices able to conduct current more easily in one direction than another. K\(_{\text{IRs}}\) are able to pass potassium better into a cell than out when measured in excised patches and thus are termed “inward rectifiers.” Under physiological conditions, the potassium concentration is greater inside the cell than out. Therefore, potassium leaves the cell through K\(_{\text{ATP}}\) channels, thus lowering the membrane potential. SURs have been shown to assemble preferentially with two K\(_{\text{IRs}}\), K\(_{\text{IR}6.1}\) and K\(_{\text{IR}6.2}\), to form large octameric channels with a \((\text{SUR}/\text{K}_{\text{IR}})^4\) stoichiometry (Clement et al., 1997; Shyng and Nichols, 1997; Inagaki et al., 1997; reviewed in Aguilar-Bryan et al., 1998, and Bryan and Aguilar-Bryan, 1999).

Several mechanisms have evolved to regulate the assembly and trafficking of the octameric channels, to insure that only those whose activity can be correctly regulated will reach the cell surface. Assembly occurs in the endoplasmic reticulum (ER); both subunits carry ER retrieval signals, an \(-\text{RKR}−\) sequence motif, which cause incompletely assembled complexes to be retained in the ER (Zerangue et al., 1999). This ER retention mechanism accounts for the original observations that SUR1 expressed alone was incompletely glycosylated (Aguilar-Bryan et al., 1995; Clement et al., 1997) and that expression of K\(_{\text{IR}6.2}\) alone failed to produce channel activity (Inagaki et al., 1995a), while expression of truncated K\(_{\text{IR}6.2}\) missing the \(-\text{RKR}−\) motif produced aberrant channels (Tucker et al., 1997).

**IV. Truncation of SUR1 Causes Severe PHHI**

The C-terminal 25 amino acids of SUR1 contain an additional peptide motif that is critical for the correct trafficking of K\(_{\text{ATP}}\) channels. This motif consists of a dileucine pair and nearby phenylalanine (-\text{XLLXXX}XFX). Removal of this motif, or substitution of alanine residues, prevents K\(_{\text{ATP}}\) channel expression at the cell surface (Sharma et al., 1999). In human SUR1, the specific sequence is -KLLSRKDSVFA. We have proposed that loss of this C-terminal signal explains
the loss of $K_{\text{ATP}}$ channel activity that is observed in PHHI $\beta$ cells with mutations that truncate SUR1 (Sharma et al., 1999).

V. Two Gene Clusters Encode the Isoforms of $K_{\text{ATP}}$ Channels

The size and chromosomal location of the human $K_{\text{ATP}}$ channel genes have been established. Two gene clusters on the short arms of human chromosomes 11 (11q15.1) and 12 (12p11.23) encode the SUR1 and KIR6.2 subunits and the SUR2 and KIR6.1 subunits, respectively (reviewed in Aguilar-Bryan and Bryan, 1999). The gene names within these clusters, using the updated nomenclature, are Sur1 = Abcc8 + KIR6.2 = KCNJ11 and Sur2 = Abcc9 + KIR6.1 = KCNJ8, respectively. Differential splicing of the terminal exon in the Sur2 gene specifies the two major SUR2A and SUR2B splice variants (reviewed in Aguilar-Bryan et al., 1998; Aguilar-Bryan and Bryan, 1999). Additional minor splice variants have been described (Chutkow et al., 1996, 1999). Based on extensive evidence (reviewed in Aguilar-Bryan et al., 1998; Aguilar-Bryan and Bryan, 1999; Babenko et al., 1998a), the SUR1/KIR6.2 pair is known to comprise the $K_{\text{ATP}}$ channels of pancreatic $\beta$ cells and also is found in neurons and neuroendocrine cells. The SUR2A/KIR6.2 pair assembles the channels in cardiac ventricular myocytes and skeletal muscle (Inagaki et al., 1996; Babenko et al., 1998b). SUR2B pairs with KIR6.1 and KIR6.2 to form the $K_{\text{ATP}}$ channels found in vascular and nonvascular smooth muscle (Isomoto et al., 1996; Yamada et al., 1997). The SUR2-based channels are the targets for a variety of potassium channel openers of potential therapeutic importance (reviewed in Challinor-Rogers and McPherson, 1994; Quast et al., 1994; Quayle and Standon, 1994; and Edwards and Watson, 1995). The Sur2/KIR6.1 gene cluster has not been connected with a disease at this time. Recent reports have suggested that mixed channels comprised of SUR1/SUR2/KIR6.1 or SUR1/SUR2/KIR6.2 subunits may exist (Miller et al., 1999; Liss et al., 1999; Gopalakrishnan et al., 1999; Yokoshiki et al., 1999). However, their pharmacologic and functional properties are not understood.

VI. Progress in Mapping Functionally Important Regions of $K_{\text{ATP}}$ Channel Subunits

$K_{\text{ATP}}$ channels have a medically important pharmacology, the $\beta$-cell channels being the classical target for hypoglycemic drugs, specifically, the first- and second-generation sulfonylureas (e.g., tolbutamide, glibenclamide, glipizide) used to control hyperglycemia in non-insulin dependent diabetes mellitus (NIDDM). $K_{\text{ATP}}$ channels are also the target for potassium channel openers (KCOs), with diazoxide being used to control hyperinsulinemic states by hyperpolarization of $\beta$ cells via channel activation. The sulfonylureas and KCOs both act through
SURs. A substantial amount of progress has been made in understanding these interactions and how they act to block and stimulate channel activity (Dickinson et al., 1997; Schwanstecher et al., 1998; Loffler-Walz and Quast, 1998; Hambrock et al., 1998,1999; Gribble et al., 1999; Babenko et al., 1999a,1999b, 1999c,2000; Meyer et al., 1999; Uhde et al., 1999; D’Hahan et al., 1999; reviewed in Bryan and Aguilar-Bryan, 1999). In addition to their differential sensitivity to sulfonylureas and KCOs, $K_{\text{ATP}}$ channel isoforms display differences in their kinetics and sensitivity to inhibitory ATP. Chimeric receptors, constructed from fragments of SUR1 and SUR2A, have been used to exploit these differences and to develop a map of the regions important in specifying 1) high-affinity sulfonylurea binding (Gribble et al., 1999; Babenko et al., 1999c; Meyer et al., 1999) 2) the action of diazoxide (Babenko et al., 2000) and other KCOs (Uhde et al., 1999; D’Hahan et al., 1999; Babenko et al., 2000); and 3) the segments that specify the differences in kinetics and sensitivity to ATP (Babenko et al., 2000). A similar chimeric strategy has been used to identify residues in the pore subunit, in the extracellular linkers after M1 and before M2, that specify the lower conductance of $K_{\text{IR}6.1}$ vs. $K_{\text{IR}6.2}$ (Repunte et al., 1999). Figure 1 provides a schematic summary of this work that we have recently reviewed (Bryan and Aguilar-Bryan, 1999).

VII. Regulation of $K_{\text{ATP}}$ Channels

Early studies on isolated, excised $\beta$-cell membrane patches demonstrated that ATP inhibited $K_{\text{ATP}}$ channels and that this inhibition was antagonized by MgADP. This discovery suggested that the link between metabolism and channel activity was through changes in the ADP/ATP ratio (Dunne and Petersen, 1986; Misler et al., 1986; Hopkins et al., 1992; Detimary et al., 1994). Analysis of missense mutations that cause PHHI has provided strong genetic support for this mechanism in the form of channels whose inhibition is not affected by ATP but that display a reduced stimulatory response to MgADP (Nichols et al., 1996; Shyng et al., 1998).

A second, quite different line of evidence in support of this mechanism comes from studies on knockout mice missing the mitochondrial uncoupling protein, UCP2 (Zhang et al., 2000). UCP2 acts to reduce the mitochondrial membrane potential, thus reducing the efficiency of conversion of ADP to ATP. At a given rate of glucose metabolism, eliminating UCP2 should decrease the ADP/ATP ratio in $\beta$ cells, thus potentiating closure of $K_{\text{ATP}}$ channels and increasing insulin output. Interestingly, UCP-2$^{-/-}$ and UCP-2$^{+/-}$ mice in the fed state have significantly higher insulin and lower blood sugar levels than control animals. At the same time, biochemical measurements indicate that ADP/ATP ratios are lower in knockout islets. In support of the knockout data, the overexpression of UCP2 in rat islets has been shown to inhibit glucose-stimulated insulin secretion (Chan et al., 1999).
FIG. 1. Summary of the functional domains and stoichiometry of $K_{ATP}$ channels. The topology of SUR is based on the model proposed by Tusnady et al. (1997); the topology of the first five helices has been defined experimentally by Raab-Graham et al. (1999). Radio-iodinated glibenclamide has been shown to photolabel an N-terminal fragment of SUR1 (Aguilar-Bryan et al., 1995; Nelson et al., 1996). The evidence for the positioning of the domains required for high-affinity sulfonylurea (tolbutamide) binding and for the action of potassium channel openers (KCOs) is covered in the text. The importance of the TMD1-5 segment of SUR1 in determining the differences in kinetics of $\beta$ cell vs. cardiac $K_{ATP}$ channel isoforms and the C-terminus of SUR1 in specifying the isoform IC$_{50}$ATP is covered in Babenko et al. (1999c). The data supporting the existence of ER retention signals, the -RKR- motifs, are in Zerangue et al. (1999). The data indicating there is a distinct trafficking signal in the C-terminus of SUR1 is in Sharma et al. (1999). The data on the differences in binding properties of the two nucleotide-binding folds (NBFs) are from Ueda et al. (1997,1999). The importance of the N-terminus of K$_{r6.2}$ in limiting bursting and for the action of tolbutamide is covered in Babenko et al. (1999a,1999b), and Koster et al. (1999). The importance of the M1 segment in SUR-K$_{r6.2}$ interactions is described in Schwappach et al. (2000). The data demonstrating that residues in the extracellular linkers specifies the differences in conductance of K$_{r6.1}$ vs. K$_{r6.2}$ channels is given in Repunte et al. (1999). The evidence for a (SUR/K$_{r6.2}$)$_4$ stoichiometry is in Clement et al. (1997), Shyng and Nichols (1997), and Inagaki et al. (1997). The location of the glycosylation sites is given in Aguilar-Bryan et al. (1995,1998) and Raab-Graham et al. (1999). Direct evidence for ATP-binding to K$_{r6.2}$ is given in Tanabe et al. (1999). The location of the binding site is not known.
While the available data are consistent with changes in the ADP/ATP ratio controlling $K_{ATP}$ channel activity, other mechanisms have been proposed. Phosphatidylinositol phosphate (PIP$_2$) has been demonstrated to reduce the sensitivity of $K_{ATP}$ channels to inhibitory ATP when applied to excised patches at high concentrations (Fan and Makielski, 1997; Shyng and Nichols, 1998; Baukrowitz et al., 1998). This suggests a potential mechanism where, at a given concentration of ATP, a local increase in β-cell PIP$_2$ would increase the number of $K_{ATP}$ channel openings. A local decrease in PIP$_2$ would reduce channel activity, thus depolarizing the β-cell membrane initiating Ca$^{2+}$ influx and potentiating insulin release. This mechanism would appear to require a PIP$_2$ decrease before the rise in Ca$^{2+}$ usually considered necessary to activate phospholipase C.

Additionally, several recent reports (Beguin et al., 1999; Lin et al., 2000) indicate that in vitro phosphorylation of KATP subunits can modulate channel activity, although the physiological significance of these observations has not been established.

VIII. PHHI vs. $K_{ATP}$ Channel Knockout Mice: Equivalent Electrophysiology, Remarkably Different Glucose Homeostasis

Electrophysiological studies on isolated β cells from three $K_{ATP}$ channel-deficient systems — the recessive form of PHHI in humans (Kane et al., 1996; Dunne et al., 1997; Otonkoski et al., 1999) and Kir6.2$^{-/-}$ (Miki et al., 1998) and Sur1$^{-/-}$ (Seghers et al., 2000) mice — have identified a common electrophysiological signature consistent with the basic model described above (see also Atwater et al., 1996; Cook and Taborsky, 1997; Aguilar-Bryan and Bryan, 1999; Mears and Atwater, 2000, for reviews). The features of this phenotype are 1) loss of K$^+$ currents activated by reduced intracellular nucleotides, as determined by whole-cell dialysis; 2) an elevated membrane potential (the cells are depolarized); 3) the presence of spontaneous, Ca$^{2+}$-dependent action potentials under low glucose conditions; and 4) increased [Ca$^{2+}$], as a result of the increased Ca$^{2+}$ influx. Figure 2 illustrates several aspects of this phenotype. In PHHI, the increase in β-cell [Ca$^{2+}$]$_i$ appears to produce an inappropriately high rate of insulin release, producing hypoglycemia. Remarkably, and unexpectedly, the $K_{ATP}$ channel null mice display normal insulin and blood sugar levels when randomly feeding, despite the presence of elevated [Ca$^{2+}$]$_i$ in isolated islets and β cells. Table 1 compares the features of PHHI neonates vs. $K_{ATP}^{-/-}$ mice.

Although the $K_{ATP}^{-/-}$ mice are normoglycemic when fed, their glucose homeostasis is impaired. Both Kir6.2$^{-/-}$ (Miki et al., 1998) and Sur1$^{-/-}$ (Seghers et al., 2000) pups display a transient hypoglycemia immediately after birth that persists for the first several days of life. The two mouse phenotypes are reported to differ subtly. The Kir6.2$^{-/-}$ mice are not significantly hypoglycemic vs. controls...
FIG. 2. (A) Whole-cell K⁺ currents in control and Sur1-knockout β cells. The K⁺ currents generated in response to a 100 msec test pulse from -70 to 60 mV, every 3 seconds, at the indicated times after membrane rupture are shown for control and Sur1-knockout β cells, respectively. As shown in the bottom plot of normalized conductance vs. time, the control currents peak at about 2 minutes, then proceed to rundown when there is no ATP in the pipette solution. No currents were detected in the Sur1-knockout β cells. Inclusion of 1 mM ATP in the pipette solution reduces the peak currents and reduces the rate of rundown significantly in the control cells. The error bars are ± S.E.
FIG. 2. (B) Sur1-knockout β cells exhibit spontaneous Ca²⁺-dependent action potentials. Records from cell-attached patches on control and Sur1-knockout β cells show spontaneous action potentials that are not inhibited by diazoxide but are suppressed by the L-type Ca²⁺ channel blocker, nifedipine.
### Table I

**PHHI Patients vs. Sur1/K<sub>ir</sub>6.2 Knockout Mice**

<table>
<thead>
<tr>
<th>Clinical</th>
<th>PHHI Patients</th>
<th>Sur1/K&lt;sub&gt;ir&lt;/sub&gt;6.2 Knockout Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Birth weight</strong></td>
<td>Large for gestational age, 3.6-5.5 kg</td>
<td>Normal, 1.47 ± 0.18 g (1.47 ± 0.20)</td>
</tr>
<tr>
<td><strong>Growth</strong></td>
<td>Normal, if diagnosed and treated early</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Fertility</strong></td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Glucose</strong></td>
<td></td>
<td></td>
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<tr>
<td>Newborn</td>
<td>Persistent hypoglycemia &lt; 20 mg/dl (50 ± 10)</td>
<td>Transient hypoglycemia Day 1, 31 ± 9 mg/dl (67 ± 10) Day 2, 53 ± 24 mg/dl Day 5, 149 ± 31 mg/dl (112 ± 9)</td>
</tr>
<tr>
<td>Adolescent</td>
<td>Hyperglycemic; high prevalence of IDDM</td>
<td>Normoglycemic when random fed, 121 ± 18 mg/dl (113 ± 17) Hypoglycemic upon fasting, 48 ± 13 mg/dl (66 ± 9)/Normal Glucose intolerant (IPGTT)</td>
</tr>
<tr>
<td><strong>Insulin</strong></td>
<td></td>
<td></td>
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<tr>
<td>Newborn</td>
<td>Inappropriate levels of insulin for hypoglycemia</td>
<td>Transient hyperinsulinemia Day 1, 86 ± 53 mU/l (38 ± 31) Day 2, 38 ± 22 mU/l Day 5, 17 ± 7 mU/l (31 ± 22)</td>
</tr>
<tr>
<td>Adolescent</td>
<td>Normal for patients in remission, absent in IDDM</td>
<td>Normal insulin levels when random fed, 25 ± 13 mU/l (27 ± 13) High insulin/blood glucose ratio upon fasting, 0.39 ± 0.17 (0.20 ± 0.08)/Normal</td>
</tr>
<tr>
<td></td>
<td>Lack of acute insulin response in patients with diffuse form of PHHI</td>
<td>Lack of acute insulin response and attenuated insulin secretion (IPGTT)</td>
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*(continued on next page)*
TABLE 1 (continued)

<table>
<thead>
<tr>
<th></th>
<th>PHHl b Cells</th>
<th>Sur1/KIR6.2 Knockout b Cells</th>
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</thead>
<tbody>
<tr>
<td><strong>K&lt;sub&gt;ATP&lt;/sub&gt; channel activity</strong></td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td><strong>Resting V&lt;sub&gt;m&lt;/sub&gt;</strong></td>
<td>Elevated with action potentials, -37.6 ± 6.7 mV (-69.4 ± 12.3 mV)</td>
<td>Elevated with action potentials, -32.6 ± 6.2 mV (-62.4 ± 12.3 mV)</td>
</tr>
<tr>
<td><strong>Resting [Ca&lt;sup&gt;2+&lt;/sup&gt;]&lt;sub&gt;i&lt;/sub&gt;</strong></td>
<td>Elevated with oscillations</td>
<td>Elevated with oscillations</td>
</tr>
<tr>
<td><strong>Insulin secretion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Basal</strong></td>
<td>Elevated</td>
<td>Normal, 52 ± 14 μU/10 islets/hr (41 ± 28)</td>
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<td><strong>Stimulation</strong></td>
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<tr>
<td><strong>Glucose</strong></td>
<td>Responds to 6-11 mM glucose</td>
<td>Lack of first-phase and attenuated second-phase (16.7 mM glucose)/No response</td>
</tr>
<tr>
<td><strong>Tolbutamide</strong></td>
<td>No response</td>
<td>No response</td>
</tr>
<tr>
<td><strong>High K&lt;sup&gt;+&lt;/sup&gt;</strong></td>
<td>No response</td>
<td>Stimulated by 30 mM KCl</td>
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</table>

[Range in parentheses represents normal values.]

after a 16-hour fast, while the Sur<sup>−/−</sup> mice are. Both knockout animals exhibit a similar mild intolerance toward intraperitoneal injections of glucose, consistent with the observed loss of first-phase insulin release in isolated islets. The fasting plasma insulin values in the K<sub>IR6.2</sub>−/− and Sur<sub>1</sub>−/− animals are lower but not significantly different (at the p < 0.05 level) from fasting control animals. In the case of the Sur<sup>1</sup>−/− animals, plasma insulin returns to control levels upon feeding, indicating insulin release is regulated in the absence of K<sub>ATP</sub> channels. Plasma insulin data have not been reported for fed K<sub>IR6.2</sub>−/− mice. In addition to being poorly able to regulate their insulin output when blood glucose falls during a fast, Sur<sup>1</sup>−/− pups do not properly regulate when blood glucose levels rise. This results in a peculiar plasma glucose profile, with the Sur<sup>1</sup>−/− mice being more hyperglycemic when glucose loaded and more hypoglycemic when fasted, relative to control animals. The two K<sub>ATP</sub>−/− animals are reported to differ markedly in their sensitivity to insulin. The K<sub>IR6.2</sub>−/− mice are reported to be insulin hypersensitive (Miki et al., 1998) secondary to loss of SUR2A/KIR6.2 channels in skeletal muscle, while we see no difference in the insulin sensitivity of the Sur<sup>1</sup>−/− mice (Seghers et al., 2000).
The secretion profiles of isolated K_{IR}6.2^{-/-} and Sur1^{-/-} islets differ subtly. The Sur1^{-/-} islets show essentially no first-phase insulin secretion in response to a shift from low (i.e., 2.8 mM) to high (i.e., 16.7 mM) glucose, while the K_{IR}6.2^{-/-} animals show a small first-phase release. By contrast, the K_{IR}6.2^{-/-} islets exhibit no second-phase release, while the Sur1^{-/-} islets show an attenuated, second-phase response, approximately 35 percent of the controls. Our hypothesis was that the K_{ATP}-independent pathway regulating secretion in the Sur1^{-/-} animals had a slow response time. In extended perifusion experiments, Sur1^{-/-} islets displayed an increase in insulin output that paralleled that seen from control islets (Figure 3A). Extended static incubation experiments demonstrated that insulin output, from both control and Sur1^{-/-} islets, was glucose dependent and could be suppressed by the Ca^{2+} channel blocker, nifedipine (Figure 3C). Furthermore, while reducing glucose in the medium resulted in a rapid return of wild-type islets to a basal secretory rate (t_{1/2} \sim 5 minutes), the Sur1^{-/-} islets were unable to rapidly reduce their insulin output (t_{1/2} \sim 67 minutes), consistent with excess insulin during fasting (Figure 3B). We have correlated the lack of first-phase insulin release with the observed impaired ability of K_{ATP} channel null mice to rapidly clear a glucose load (i.e., their mild glucose intolerance) and hypothesize that the greater hypoglycemia upon fasting is the result of their inability to shutdown insulin release when glucose levels fall. These arguments are summarized in a generalized model in Figure 4A.

Since the insulin secretory responses to both the rise and fall of glucose are blunted in the knockout animals, these results suggested that while the mean values of blood glucose may be equivalent, the variance (\sigma) of measured values in randomly feeding animals should be greater. In short, wild-type animals should display a much-tighter control of blood glucose than the knockout animals. Figure 4B illustrates a series of measurements consistent with the hypothesis in which wild-type and Sur1-knockout animals were given constant access to food and glucose measurements were carried out over an extended period. We attempted to "stress" the animals by increasing the sugar content of their diet through supplementing the drinking water with dextrose. The data indicate that the mean values of the wild-type and knockout animals are the same — that increasing glucose in the drinking water had no clear effect and that the standard deviations (error bars) of the knockout values are significantly greater than for the wild type. As a measure of this increased variation, we averaged all of the control and knockout values. The values were 8.55 \pm 1.67 vs. 8.41 \pm 3.19 mM for the control (n = 5) and Sur1-knockout (n = 6) animals, respectively. The values for the variance are 2.77 vs. 10.2 for the control and knockout animals, respectively.
FIG. 3. Comparison of the insulin secretory response of control vs. Sur1-knockout islets. (A) Perifusion experiments that assess insulin output from isolated pancreatic islets as a function of time demonstrate that Sur1-knockout islets lack first-phase insulin secretion and display an attenuated second-phase response to elevated glucose. Note the break in the time line at 25 minutes. The longer time values are the mean values of seven 1-minute time points flanking the given time, the error bars are ± S.D. (B) At the end of the perifusion experiment shown in (A), the islets were switched to low glucose to assess their rates of return to basal secretory levels. For purposes of comparison, we have normalized the data to an initial value of 1 by dividing by the average value immediately before switching to 2.8 mM glucose. Note that the absolute values of insulin output from control islets fall below that of the Sur1−/− islets in approximately 5 minutes. The results from two separate experiments are plotted; the lines are the best fits of a monoeponential function to the combined data sets. The glucose concentrations in the perfusing medium in (A) and (B) are indicated by the bars. (C) Static incubation experiments demonstrate that the Sur1 islets display glucose-stimulated insulin secretion. Islets were cultured for 20 hours at the indicated glucose concentrations before assay of insulin released into the culture medium. At 16.7 mM glucose, the values were 494.5 ± 88.9 vs. 285.1 ± 62.0 ng/ml/10 islets/20 hr (n = 10; mean ± S.D.), respectively. The lines are best fits to a logistic equation; the half-maximal values are 9.0 and 9.8 mM glucose for the control and Sur1−/− groups, respectively. For comparative purposes, the mean values of blood glucose was 6.5 ± 0.99 mM (n = 77) and did not differ significantly for randomly fed control vs. Sur1−/− animals. The + nifedipine samples (1 μM) were incubated with the highest concentration of glucose tested (16.7 mM). The error bars are ± S.D. [Data from Seghers, V., Nakazaki, M., DeMayo, F., Aguilar-Bryan, L., and Bryan, J. J. Biol. Chem. 275, 9270-9277, 2000.]
FIG. 4. Generalized insulin secretory responses and consequent blood glucose levels in Surl-knockout mice. The top panel in (A) illustrates hypothetical changes in glucose concentration as the result of glucose infusion into mice or through a change in the perfusion medium in isolated islet experiments. The middle panel illustrates the resulting insulin secretory response from control (solid line) or Surl-knockout islets (dotted line), in response to the shifts in glucose stimulation. The lower panel in (A) illustrates the proposed changes in blood glucose levels in response to insulin output. The loss of first-phase secretion in the Surl-knockout animals results in higher peak glucose levels and slower clearance of the glucose load. The reduced insulin output from the Surl-knockout islets would result in a higher steady-state glucose level. The impaired ability of the Surl-knockout islets to restrict insulin output during a fall in glucose concentration leads to more-pronounced hypoglycemia in the knockout animals, for example, during fasting. (B) The generalized results shown in (A) suggested that randomly feeding animals should display a wider variation in their blood glucose values. The results shown here are consistent with this hypothesis. Control (5) and knockout (6) animals were allowed free access to normal mouse chow; the drinking water was supplemented with glucose as indicated. Blood glucose values were taken at the indicated times. The mean values ± S.D. are plotted for control and Surl-knockout animals. There was no significant effect of the additional glucose on the mean blood glucose levels but the knockout animals display an increased variation in their glucose levels vs. controls.
A comparison of the features of PHHI β cells and neonates vs. mice missing K<sub>ATP</sub> channels indicates both have an equivalent β-cell signature. However, their insulin secretory responses and blood glucose values differ. Both the K<sub>IR6.2</sub>−/− and the S<sub>ur1</sub>−/− animals are hypoglycemic at birth but develop a compensatory mechanism to maintain normoglycemia. (We will restrict the specifics of this discussion to the S<sub>UR1</sub> null animals, as Miki et al., 1998, have argued an alternative mechanism for the K<sub>IR6.2</sub> null mice based on insulin hypersensitivity.) The S<sub>ur1</sub>−/− pups resolve their hypoglycemia within 2 to 3 days (the K<sub>IR6.2</sub>−/− pups display a similar pattern), while, for unknown reasons, PHHI neonates do not. Compensation requires that the knockout islets become refractory to elevated [Ca<sup>2+</sup>]i and “acquire” a K<sub>ATP</sub>-independent mechanism for regulation of insulin release. It is not clear if the latter is always operative, perhaps during second-phase insulin release, or develops as a result of the loss of K<sub>ATP</sub> channels. We have pointed out that the pattern of insulin release in the S<sub>ur1</sub>−/− mice is similar to the K<sub>ATP</sub>-independent, Ca<sup>2+</sup>- and glucose-dependent potentiation of insulin release observed from isolated islets by several groups (Panten et al., 1988; Best et al., 1992; Gembal et al., 1992; Straub et al., 1998a). However, it differs from the GTP-dependent, Ca<sup>2+</sup>-independent potentiation observed upon simultaneous activation of protein kinases A and C (Komatsu et al., 1995, 1997a, 1997b; Straub et al., 1998b) (see Aizawa et al., 1998, for a brief review of this area). We have begun to determine whether the S<sub>ur1</sub> knockout animals retain sensitivity to other factors known to stimulate insulin release (Newgard and McGarry, 1995; Prenki, 1996; Sato and Henquin, 1998; Lang, 1999). Our preliminary comparison of the responsiveness of isolated islets to agents (i.e., IBMX, forskolin) that elevate cyclic AMP (cAMP) levels, thus activating cAMP-dependent protein kinases (PKA) and markedly stimulating glucose-induced insulin release from wild-type islets, shows the S<sub>ur1</sub> knockout islets are poorly responsive to small changes (two- to five-fold) in cAMP but do respond to very high concentrations (> 100-fold increase). The S<sub>ur1</sub>−/− islets also display a reduced insulin secretory response to carbachol, although phorbol esters like tetradecanoyl phorbol acetate (TPA), known to activate protein kinase C, can potentiate glucose-stimulated insulin secretion equally from both wild-type and S<sub>ur1</sub>−/− islets (Nakazaki et al., in preparation). The results are consistent with a reduced state of phosphorylation of protein(s) critical for insulin release. Alternatively, our results could be explained by an impairment in the cAMP-stimulated pathway that involves members of the novel Epac family of cAMP-dependent RapGEFs (Kawasaki et al., 1998; de Rooij et al., 1998). These proteins, Epac1 (cAMP-GEFI) and Epac2 (cAMP-GEFII), are closely related guanine-nucleotide exchange factors for the small GTPases Rap1 and Rap2 (de Rooij et al., 2000). Rap1 has been implicated in insulin secretion (Leiser et al.,
1995; Kowluru et al., 1997). Clearly, myriad reasons could account for the impaired response of Sur1-/- islets to these various agents. With the available data, we cannot distinguish between possible mechanisms but hypothesize that the impaired responsiveness develops during the first few days of life in response to elevated cytosolic calcium and accounts for the resolution of the early hypoglycemia observed in the knockout animals.

X. Summary

The availability of cloned cDNAs encoding the subunits that comprise members of the K\textsubscript{ATP} channel family continues to provide insight into their structure and function. Studies over the past 5 years have illuminated the stoichiometry, biophysics, and pharmacology of K\textsubscript{ATP} channels and have identified functionally important regions of both subunits. Additionally, it is now clear that trafficking plays a major role in the correct assembly and surface expression of these channels. Endoplasmic reticulum retention/retrieval signals have been defined on both SUR1 and K\textsubscript{IR}6.2 that insure that only completely assembled channels (SUR1/K\textsubscript{IR}6.2)\textsubscript{4} reach the cell surface. An additional motif has been identified in SUR1 that is required for surface expression and, when removed as a result of mutation, produces PHHI. The study of the functional properties of missense mutations that cause PHHI has provided insight into the mechanism by which the activity of these channels is coupled to glucose metabolism. Although PHHI neonates lacking K\textsubscript{ATP} channel activity are profoundly hypoglycemic, as a result of inappropriately high insulin secretion, recent work on knockout mice lacking these channels indicates that they are able to compensate using an as-yet-undefined, K\textsubscript{ATP}-independent regulatory mechanism to maintain near-normal insulin and blood sugar levels. K\textsubscript{ATP}-independent regulation in the knockout mice is glucose and Ca\textsuperscript{2+} dependent and is presumably equivalent to recently described K\textsubscript{ATP}-independent regulatory pathways in isolated pancreatic islets. The compensatory mechanism(s) are not known but preliminary results indicate that the loss of these channels and subsequent chronic increase in cytosolic calcium levels produce change beyond the simple loss of plasma membrane K\textsuperscript{+} channels. Understanding these compensatory changes and learning how to activate them in PHHI neonates could provide a novel therapeutic approach for the treatment of this disorder.

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