Of Mice and Men: K_{ATP} Channels and Insulin Secretion

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ABSTRACT

K_{ATP} channels are a unique, small family of potassium (K^+)-selective ion channels assembled from four inward rectifier pore-forming subunits, $K_{IR}6.x$, 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 β cells in the islets of Langerhans, K_{ATP} channels are part of an ionic mechanism that couples glucose metabolism to insulin secretion. This chapter 1) briefly describes the properties of K_{ATP} channels; 2) discusses data on a genetically recessive form of persistent hyperinsulinemic hypoglycemia of infancy (PHHI), caused by loss of β-cell K_{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_{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 β 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 β cells in the islets of Langerhans within the pancreas. While the molecular mechanism(s) of insulin resistance and β-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-

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tion in nontumor states are less well understood. During the last half-dozen years, insight into the genetic basis of congenital hyperinsulinism, or persistent hyper-insulinemic 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 $[Ca^{2+}]_i$. 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 nesidioblas-tosis (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 hypo-glycemia (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 sub-classes 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 K_{ATP} channels (Inagaki *et al.*, 1995a). These mutations cause PHHI, termed HI-SUR1 (OMIM 600509) or HI-$K_{IR}6.2$ (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 $[Ca^{2+}]_i$. 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_{IR}s

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_{ATP} channels, although it does not power K^+ movement.

The regulatory subunits are termed SURs because they bind hypoglycemic sulfonylureas. The β-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_{IR}s, 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_{IR}s 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_{ATP} channels, thus lowering the membrane potential. SURs have been shown to assemble preferentially with two K_{IR}s, $K_{IR}6.1$ and $K_{IR}6.2$, to form large octameric channels with a $(SUR/K_{IR}6.x)_4$ 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 *–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 $K_{IR}6.2$ alone failed to produce channel activity (Inagaki *et al.,* 1995a), while expression of truncated $K_{IR}6.2$ missing the *–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_{ATP} channels. This motif consists of a dileucine pair and nearby phenylalanine (*-XLLXXXXXXFX*). Removal of this motif, or substitution of alanine residues, prevents K_{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_{ATP} channel activity that is observed in PHHI β cells with mutations that truncate SUR1 (Sharma *et al.*, 1999).

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

The size and chromosomal location of the human K_{ATP} channel genes have been established. Two gene clusters on the short arms of human chromosomes 11 (11p15.1) and 12 (12p11.23) encode the SUR1 and $K_{IR}6.2$ subunits and the SUR2 and $K_{IR}6.1$ subunits, respectively (reviewed in Aguilar-Bryan and Bryan, 1999). The gene names within these clusters, using the updated nomenclature, are Sur1 = Abcc8 + $K_{IR}6.2$ = KCNJ11 and Sur2 = Abcc9 + $K_{IR}6.1$ = 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/$K_{IR}6.2$ pair is known to comprise the K_{ATP} channels of pancreatic β cells and also is found in neurons and neuroendocrine cells. The SUR2A/$K_{IR}6.2$ pair assembles the channels in cardiac ventricular myocytes and skeletal muscle (Inagaki *et al.*, 1996; Babenko *et al.*, 1998b). SUR2B pairs with $K_{IR}6.1$ and $K_{IR}6.2$ to form the K_{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/$K_{IR}6.1$ gene cluster has not been connected with a disease at this time. Recent reports have suggested that mixed channels comprised of SUR1/SUR2/$K_{IR}6.1$ or SUR1/SUR2/$K_{IR}6.2$ 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_{ATP} Channel Subunits

K_{ATP} channels have a medically important pharmacology, the β-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_{ATP} channels are also the target for potassium channel openers (KCOs), with diazoxide being used to control hyperinsulinemic states by hyperpolarization of β 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_{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 $K_{IR}6.1$ vs. $K_{IR}6.2$ (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_{ATP} Channels

Early studies on isolated, excised β-cell membrane patches demonstrated that ATP inhibited K_{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 β cells, thus potentiating closure of K_{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).
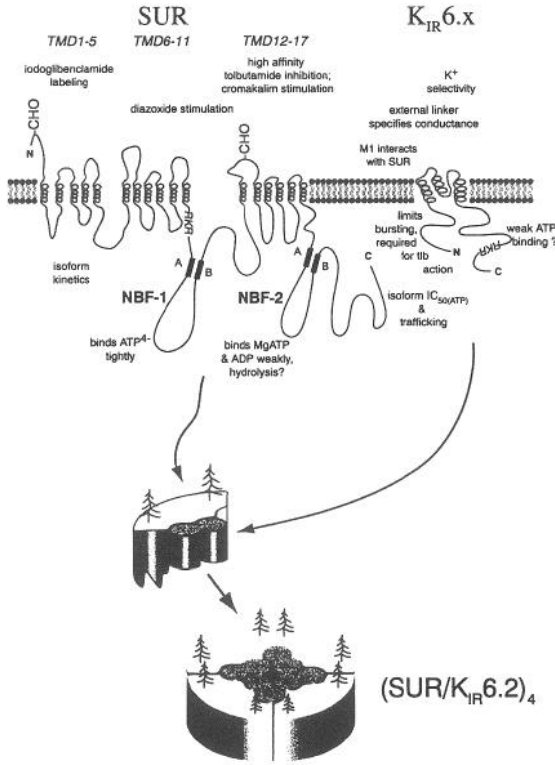
FIG. 1. Summary of the functional domains and stoichiometry of K_{ATP} channels. The topology of SUR is based on the model proposed by Tusnady *et al.* (1997); the topology of the first five helices has been defined experimentally by Raab-Graham *et al.* (1999). Radio-iodinated glibenclamide has been 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 β 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 $K_{IR}6.2$ 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-$K_{IR}6.2$ interactions is described in Schwappach *et al.* (2000). The data demonstrating that residues in the extracellular linkers specifies the differences in conductance of $K_{IR}6.1$ vs. $K_{IR}6.2$ channels is given in Repunte *et al.* (1999). The evidence for a (SUR1/$K_{IR}6.2)_4$ 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 $K_{IR}6.2$ 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. Phosphatidyinositol 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 K_{ATP} 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 $K_{IR}6.2^{-/-}$ (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 $[Ca^{2+}]_i$ 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 I 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 $K_{IR}6.2^{-/-}$ (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 $K_{IR}6.2^{-/-}$ mice are not significantly hypoglycemic vs. controls
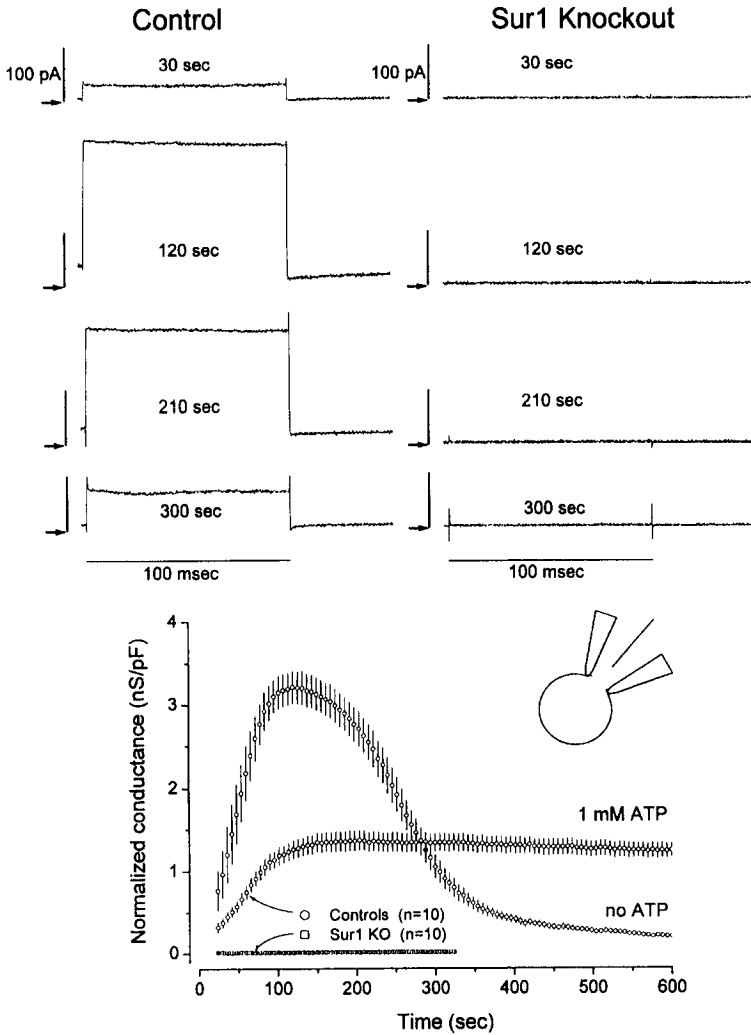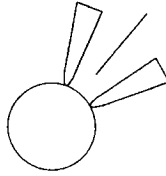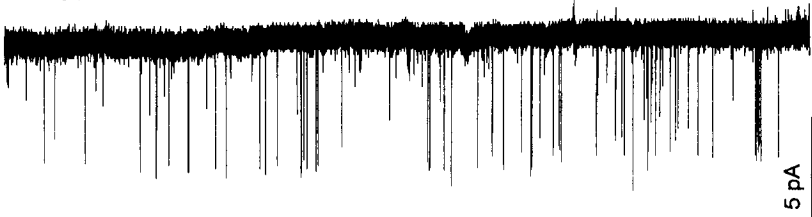
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Whole Cell Mode

Control   Sur1 Knockout

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.
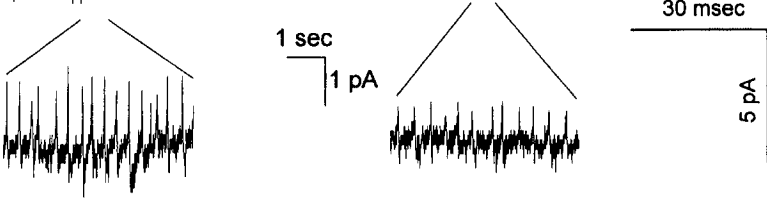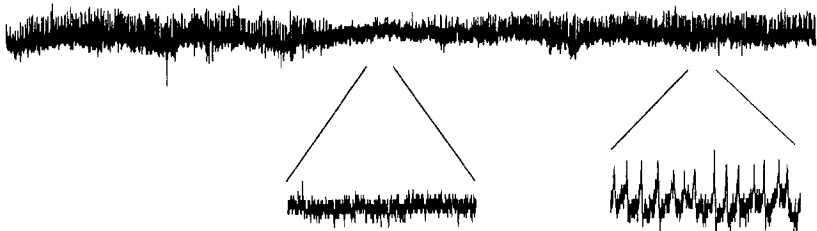
FIG. 2. (B) Sur1-knockout β cells exhibit spontaneous Ca²⁺-dependent action potentials. Records from cell-attached patches on control and Sur1-knockout β cells show spontaneous action potentials that are not inhibited by diazoxide but are suppressed by the L-type Ca²⁺ channel blocker, nifedipine.

TABLE I
PHHI Patients vs. K_{ATP} Channel Knockout Mice

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

TABLE I *(continued)*

	Basic	
	PHHI β Cells	Sur1/K$_{IR}$6.2 Knockout β Cells
K$_{ATP}$ channel activity	Absent	Absent
Resting Vm	Elevated with action potentials	Elevated with action potentials, -32.6 ± 6.2 mV (-62.4 ± 12.31)
Resting [Ca^{2+}]$_i$	Elevated with oscillations	Elevated with oscillations
Insulin secretion		
Basal	Elevated	Normal, 52 ± 14 μU/ 10 islets/hr (41 ± 28)
Stimulation		
Glucose	Responds to 6-11 nM glucose	Lack of first-phase and attenuated second-phase (16.7 mM glucose)/ No response
Tolbutamide	No response	No response
High K$^+$	No response	Stimulated by 30 mM KCl

[Range in parentheses represents normal values.]

after a 16-hour fast, while the Sur1$^{-/-}$ 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$_{IR}$6.2$^{-/-}$ and Sur1$^{-/-}$ animals are lower but not significantly different (at the $p < 0.05$ level) from fasting control animals. In the case of the Sur1$^{-/-}$ animals, plasma insulin returns to control levels upon feeding, indicating insulin release is regulated in the absence of K$_{ATP}$ channels. Plasma insulin data have not been reported for fed K$_{IR}$6.2$^{-/-}$ mice. In addition to being poorly able to regulate their insulin output when blood glucose falls during a fast, Sur1$^{-/-}$ pups do not properly regulate when blood glucose levels rise. This results in a peculiar plasma glucose profile, with the Sur1$^{-/-}$ mice being more hyperglycemic when glucose loaded and more hypoglycemic when fasted, relative to control animals. The two K$_{ATP}$$^{-/-}$ animals are reported to differ markedly in their sensitivity to insulin. The K$_{IR}$6.2$^{-/-}$ mice are reported to be insulin hypersensitive (Miki *et al.,* 1998) secondary to loss of SUR2A/K$_{IR}$6.2 channels in skeletal muscle, while we see no difference in the insulin sensitivity of the Sur1$^{-/-}$ 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 (σ) 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 ± 1.67 vs. 8.41 ± 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.
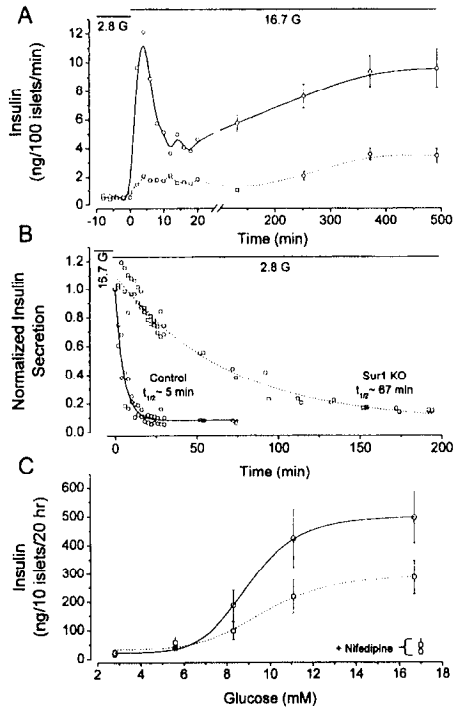
FIG. 3. Comparison of the insulin secretory response of control vs. Sur1-knockout islets. (A) Perifusion experiments that assess insulin output from isolated pancreatic islets as a function of time demonstrate that Sur1-knockout islets lack first-phase insulin secretion and display an attenuated second-phase 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 monoexponential 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 6.530.99 mM (n = 72) 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.]
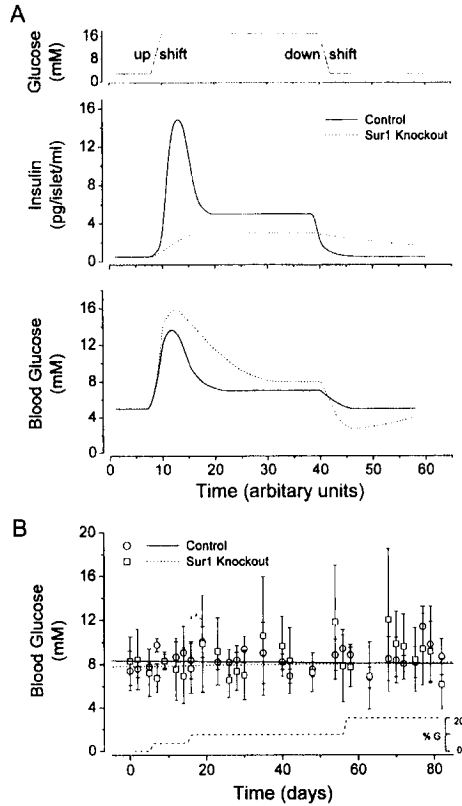
FIG. 4. Generalized insulin secretory responses and consequent blood glucose levels in Sur1-knockout mice. The top panel in (A) illustrates hypothetical changes in glucose concentration as the result of glucose infusion into mice or through a change in the perifusion medium in isolated islet experiments. The middle panel illustrates the resulting insulin secretory response from control (solid line) or Sur1-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 Sur1-knockout animals results in higher peak glucose levels and slower clearance of the glucose load. The reduced insulin output from the Sur1-knockout islets would result in a higher steady-state glucose level. The impaired ability of the Sur1-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 Sur1-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.

IX. Compensation for Loss of K_{ATP} Channels
May Occur Shortly After Birth

A comparison of the features of PHHI β cells and neonates vs. mice missing K_{ATP} channels indicates both have an equivalent β-cell signature. However, their insulin secretory responses and blood glucose values differ. Both the $K_{IR}6.2^{-/-}$ and the $Sur1^{-/-}$ animals are hypoglycemic at birth but develop a compensatory mechanism to maintain normoglycemia. (We will restrict the specifics of this discussion to the SUR1 null animals, as Miki et al., 1998, have argued an alternative mechanism for the $K_{IR}6.2$ null mice based on insulin hypersensitivity.) The $Sur1^{-/-}$ pups resolve their hypoglycemia within 2 to 3 days (the $K_{IR}6.2^{-/-}$ pups display a similar pattern), while, for unknown reasons, PHHI neonates do not. Compensation requires that the knockout islets become refractory to elevated $[Ca^{2+}]_i$ and "acquire" a K_{ATP}-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_{ATP} channels. We have pointed out that the pattern of insulin release in the $Sur1^{-/-}$ mice is similar to the K_{ATP}-independent, Ca^{2+}- 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^{2+}-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 Sur1 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 Sur1 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 $Sur1^{-/-}$ islets also display a reduced insulin secretory response to carbachol, although phorbol esters like trtradecanoyl phorbol acetate (TPA), known to activate protein kinase C, can potentiate glucose-stimulated insulin secretion equally from both wild-type and $Sur1^{-/-}$ 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_{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_{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 $K_{IR}6.2$ that insure that only completely assembled channels $(SUR1/K_{IR}6.2)_4$, 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_{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_{ATP}-independent regulatory mechanism to maintain near-normal insulin and blood sugar levels. K_{ATP}-independent regulation in the knockout mice is glucose and Ca^{2+} dependent and is presumably equivalent to recently described K_{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^+ 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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