K\textsubscript{ATP} channels and insulin secretion disorders

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Huopio, H., S.-L. Shyng, T. Otonkoski, and C. G. Nichols. K\textsubscript{ATP} channels and insulin secretion disorders. Am J Physiol Endocrinol Metab 283: E207–E216, 2002; 10.1152/ajpendo.00047.2002.—ATP-sensitive potassium (K\textsubscript{ATP}) channels are inhibited by intracellular ATP and activated by ADP. Nutrient oxidation in \(\beta\)-cells leads to a rise in [ATP]-to-[ADP] ratios, which in turn leads to reduced K\textsubscript{ATP} channel activity, depolarization, voltage-dependent Ca\textsuperscript{2+} channel activation, Ca\textsuperscript{2+} entry, and exocytosis. Persistant hyperinsulinemic hypoglycemia of infancy (HI) is a genetic disorder characterized by dysregulated insulin secretion and, although rare, causes severe mental retardation and epilepsy if left untreated. The last five or six years have seen rapid advance in understanding the molecular basis of K\textsubscript{ATP} channel activity and the molecular genetics of HI. In the majority of cases for which a genotype has been uncovered, causal HI mutations are found in one or the other of the two genes, SUR1 and Kir6.2, that encode the K\textsubscript{ATP} channel. This article will review studies that have defined the link between channel activity and defective insulin release and will consider implications for future understanding of the mechanisms of control of insulin secretion in normal and diseased states.

ATP-sensitive potassium; hyperinsulinemic hypoglycemia of infancy; pancreas; Kir6.2; diabetes; SUR1

INTRODUCTION: THE INSULIN SECRETION PARADIGM

ATP-sensitive potassium (K\textsubscript{ATP}) channels are a unique class of potassium channels with the hallmark physiological properties of being inhibited by intracellular ATP and activated by ADP. Their role in regulation of insulin secretion from the islets of Langerhans has long been recognized (5). Decreased blood glucose leads to a fall in \(\beta\)-cell glucose concentration and, hence, a decreased [ATP]-to-[ADP] ratio. This opens K\textsubscript{ATP} channels, causing hyperpolarization of the cell and consequent closure of calcium (Ca\textsuperscript{2+}) channels, block of Ca\textsuperscript{2+} entry, and suppression of insulin secretion (Fig. 1A). However, there have been lingering arguments that this straightforward scenario cannot account for the entire picture of insulin secretion. In particular, insulin secretion occurs in two phases, an early transient phase and a secondary sustained phase (24). In isolated pancreatic islets, maximal depolarization of the cells by exposure to high [K\textsuperscript{+}] causes only a transient insulin release; under conditions in which K\textsubscript{ATP} channels may be continuously activated by diazoxide, a glucose-dependent release of insulin can still be seen (18).

Thus a concept is developing that K\textsubscript{ATP}-dependent inhibition of Ca\textsuperscript{2+} entry and vesicle release is the major mechanism for regulating insulin secretion, but that K\textsubscript{ATP}-independent mechanisms must modulate it or provide alternative pathways for controlling insulin secretion (4). In this regard, recent animal studies, in which the K\textsubscript{ATP} channel proteins have been directly manipulated, have raised more questions than they have answered (see ANIMAL MODELS OF HI). Nevertheless, the weight of evidence from studies of the genetic basis of hypoglycemic hyperinsulinemia provides compelling evidence for a critical role of K\textsubscript{ATP} in linking nutrient levels to insulin secretion. The purpose of this article is to review the extensive work of the last few years that has defined this link and to consider unanswered questions and implications for future under-
standing of the mechanisms of control of insulin secretion in normal and clinical conditions.

CONGENITAL HYPERINSULINEMIA, OR PERSISTENT HYPERINSULINEMIC HYPOGLYCEMIA OF INFANCY: A BREAKDOWN IN THE REGULATION OF INSULIN SECRETION

Persistent hyperinsulinemic hypoglycemia of infancy (HI), also referred to as congenital hyperinsulinism, is a genetic disorder characterized by dysregulated insulin secretion (8) and is the most common cause of persistent and recurrent hypoglycemia in infancy. The typical incidence of the disease is estimated to be 1:50,000 live births, but in some areas of high consanguinity, it is as high as 1:3,000 (10, 34, 36). The clinical phenotype is variable, but the disease can be a major cause of severe mental retardation and epilepsy if not treated properly (6, 37, 38). Due to the anabolic effect of insulin, the newborn with HI may be macrosomic at birth, thus resembling an infant of a diabetic mother reflecting the prenatal hyperinsulinism; in most cases, symptoms of hypoglycemia (such as floppiness, jitteriness, poor feeding, and lethargy) appear during the first postnatal hours or days but in a few cases during the first year. The diagnosis of HI is based on the detection of 1) nonketotic hypoglycemia, 2) inappropriately high (measurable) insulin concentration and raised C-peptide at the point of hypoglycemia, and 3) increased glucose requirements to maintain normoglycemia. Low levels of serum free fatty acids and ketone bodies due to the antilipolytic effect of insulin at the time of hypoglycemia, as well as the glycemic response to glucagon administration, support the diagnosis of hyperinsulinism (7).

The KATP channel opener diazoxide, administered together with chlorothiazide, is the mainstay of medical management of HI, and hormones like somatostatin and glucagon are also of proven benefit. However, some patients fail to respond to medical treatment, necessitating surgical removal of up to 95% of the pancreas to avoid permanent neurological damage (7, 60). The responsivity of some patients to drugs that activate KATP channels is consistent with the proposed role of KATP channels in controlling insulin secretion and with a relative deficiency of KATP channel activity in these patients. Over the last five years, there has been rapid advance in the molecular genetics of HI. Although several mutations have been uncovered in genes encoding metabolic enzymes (glucokinase, glutamate dehydrogenase), and the molecular basis remains to be established in 50% of all cases, the majority of causal mutations that have been uncovered are in the genes encoding the KATP channel.

HI MUTATIONS IN THE KATP CHANNEL GENES SUR1 AND Kir6.2: THE MOLECULAR BASIS OF THE DISEASE

Since the 1940s, it has been realized that binding of sulfonylureas to a receptor in the β-cell membrane can
trigger insulin secretion, and in the early 1980s it was demonstrated that [3H]glibenclamide labeled an ~140-kDa protein in the membranes of insulin-secreting cells. A subsequent determined purification of the receptor resulted in the cloning of SUR1 (3), which generated $K_{\text{ATP}}$ channels when coexpressed with the cloned K channel subunit Kir6.2 (27) (Fig. 1B). The channels are normally formed as an octamer consisting of four Kir6.2 subunits that generate the pore and four SUR1 subunits (12, 28, 58). Although Kir6.2 tetramers can generate channels in the absence of SUR1 (70), each subunit carries an endoplasmic reticulum (ER) retention signal that is shielded when complexed with the other subunit, allowing ER export and the generation of channel activity at the surface membrane (74). As we will consider, this multimeric nature of the functional channel allows for the generation of complex genotype-phenotype correlations when subunit mutations are present heterozygously, as is likely in many HI patients.

Regulation of channel activity involves complex interactions with cytosolic nucleotides. Hallmark ATP inhibition occurs through nonhydrolytic interaction with the cytoplasmic domains of the Kir6.2 subunit (14, 32, 56, 63, 68–70) (Fig. 1C). Physiological activation probably results from the counteracting effect of ATP hydrolysis and MgADP binding occurring at SUR1 (5) (Fig. 1C). SUR1 is a member of the ATP binding cassette (ABC) family of membrane proteins, each of which contains two classical nucleotide binding folds (NBFs) (24a), capable of nucleotide binding and hydrolysis (75) (Fig. 1C). Examination of an HI disease-causing mutation (G1479R) in NBP2 (45) provided the first clue to the role of SUR1 in channel activation. This mutation selectively abolished MgADP (and diazoxide) stimulation of channel activity, with no effect on ATP inhibition. Similar results are obtained with multiple other introduced mutations in SUR1 (22, 57), consistent with nucleotide hydrolysis at the SUR NBFs being involved in channel stimulation by MgADP. Ueda et al. (71) showed that ATP binds to NBF1 and that this binding is stabilized by MgADP binding to NBF2 (72). Both NBFs of SUR2A are capable of hydrolyzing ATP (9), and mutations that reduce the ATPase activity produce channels with increased ATP sensitivity, consistent with a model whereby SUR acts as a “hypersensitivity switch” to modulate ATP sensitivity of channel activity (57, 75, 76), the hypersensitizing switch being turned off by ATP hydrolysis at the NBFs, with MgADP binding as a product analog, to stabilize the “activated” state (Fig. 1C).

Both SUR1 and Kir6.2 were localized to human chromosome 11p15.1, and coincident with channel cloning efforts, a number of groups traced the genetic locus of HI to the same region (3, 19, 66). HI-associated SUR1 mutations were rapidly identified (67), and more than 50 HI mutations have now been recognized in the SUR1 and Kir6.2 genes (55). Most $K_{\text{ATP}}$ channel defects resulting from HI mutations can be divided into two major functional categories: defects of expressed channel properties, and biosynthetic or trafficking defects that lead to lack of, or reduced, surface expression of channels. The first mutation shown to alter $K_{\text{ATP}}$ channel properties was an HI-associated missense mutation (G1479R) in the NBP2 of SUR1 (45) (Fig. 1B). Recombinant channels encoded by this mutation in hamster SUR1 behave essentially normally with respect to single channel conductance and ATP sensitivity in inside-out membrane patches. However, these channels do not respond to stimulation by MgADP, rendering the channels “physiologically nonfunctional” because they cannot open in response to a rise in [ADP] after glucose deprivation (45). As a result, β-cells are expected to remain depolarized, and insulin secretion is expected to continue. Reduced sensitivity to stimulation by MgADP is a defect of channels generated by a number of HI-associated SUR1 mutations, including F591L, T1139M, R1215Q, G1382S, and E1506K (25, 59) (Fig. 1B). These results suggest that a defective MgADP response is a common mechanism of HI and demonstrate the critical role of MgADP in activating $K_{\text{ATP}}$ channels in physiological conditions.

Alteration of channel response to MgADP by HI mutations could be due to direct effects on nucleotide binding and hydrolysis at the NBFs or to defects in the subsequent coupling events that lead to channel opening. One HI mutation (SUR1[R1420C]) lowers the affinity of NBF2 for ATP and ADP and abolishes the cooperative binding between the two NBFs (36). Curiously, this biochemical defect was not immediately reflected in the EC50 for MgADP activation of channel activity when wild-type Kir6.2 was used for channel reconstitution, but because MgADP stimulates channel activity on one hand by interaction with SUR1, and inhibits channel activity on the other by interaction with Kir6.2, subtle differences in MgADP dose response may be masked. To separate these two opposing effects, Matsuo et al. (36) also examined SUR1 expressed with Kir6.2[R50G], a mutation that has greatly reduced sensitivity to ATP inhibition. Consistent with the biochemical data, the EC50 for MgADP activation of the SUR1[R1420C] + Kir6.2[R50G] mutant channel is about three times higher than that of wild-type SUR1 + Kir6.2[R50G] channels. Biochemical studies like this help map out the residues that are critical for direct nucleotide interaction and those that are involved in the functional coupling between SUR1 and Kir6.2.

A second major consequence of HI mutations in $K_{\text{ATP}}$ subunits is reduced, or lack of, surface expression of channels. This is obvious for mutations that cause large truncation of SUR1 or Kir6.2 proteins. Mutations in the Kir6.2 gene, which is located five kilobases downstream of the SUR1 gene, actually seem to be relatively rare causes of HI, but the nonsense mutation Y12X, which leads to truncation of the protein after 12 amino acids, was detected in a single homozygous patient of a Palestinian Arab family (43). Two other recessively inherited HI-associated mutations in Kir6.2 have thus far been reported. A homozygous point mutation (L147P) is associated with a severe and drug-resistant form of HI in a patient of Iranian origin.
(65), and a third Kir6.2 mutation (W91R) was identified in a newborn of Palestinian descent with severe disease that required pancreatectomy (2). None of these mutations generated active K\textsubscript{ATP} channels when coexpressed with wild-type SUR1. In the first case this is clearly due to lack of channel protein, but the mechanism of the latter two remains unknown, possibly reflecting altered trafficking or abolition of ion conductance. Two additional Kir6.2 mutations have recently been detected in one Finnish compound heterozygous patient (Huopio, H., unpublished observations). The first mutation is located before the translation start site and forms a new start codon and a frame shift. The other mutation causes an amino acid change from lysine to asparagine. The patient had a very severe form of HI and was treated with subtotal pancreatectomy at the age of 11 days.

A mutation in exon 35 of SUR1 causes a frame shift after R1437, resulting in a protein with an additional 23 extraneous amino acids and deletion of NBF2 (16). When coexpressed with Kir6.2 in COS cells, the mutant SUR1 again fails to generate active channels. One potential explanation is that truncation of NBF2 might cause defective trafficking of the channel complex, because it also removes an anterograde traffic signal encoded in the COOH terminus (54). However, Sakura et al. (48) reported that an SUR1 splice variant that introduced a frame shift after amino acid 1,330 did generate K\textsubscript{ATP} current when coexpressed with Kir6.2 in Xenopus oocytes. In this case, NBF2 is also completely absent, but there is an addition of 25 novel amino acids after residue 1,330. The discrepancy between the two cases may be due to the nature of the additional amino acids introduced by the frame shift, but it is also possible that the requirements for trafficking and surface expression are different in Xenopus oocytes and mammalian cells.

**TRAFFICKING DEFECTS IN HI**

A number of mutant proteins that do not express in mammalian cells are expressed in Xenopus oocytes, an example being the common cystic fibrosis-causing mutation (ΔF508) in the cystic fibrosis transmembrane regulator (CFTR), another ABC protein related to SUR1 (15). Cartier et al. (11) recently demonstrated that the mutation responsible for ~20% of Ashkenazi Jewish HI (SUR1[ΔF1388]) causes both defective trafficking and lack of surface expression of functional K\textsubscript{ATP} channels and altered channel function (Fig. 2A). The mutant protein appears to be retained in the ER, like the CFTR[ΔF508] mutation. Partridge et al. (47) subsequently reported that another single amino acid mutation (R1394H) also causes defective trafficking in mammalian cells, but rather than being retained in the ER, the protein appears to accumulate in the Golgi. Interestingly, this defect is also not observed when the mutant is expressed in Xenopus oocytes.

The finding that defective K\textsubscript{ATP} channel trafficking is a molecular basis of HI highlights the importance of understanding how trafficking and surface expression of channels are regulated. Proper cell surface expression...
sion of $K_{ATP}$ channels is thought to be under the control of a tripeptide ER retention signal (RKR) that is present in both SUR1 and Kir6.2 subunits (74). When expressed independently, each protein is normally retained in the ER due to exposure of the RKR signal; removal of the retention signal allows either protein to escape the ER quality control mechanism and express on the cell surface (70, 74). The association of SUR1 and Kir6.2 is proposed to mutually shield the ER retention signals and permit the channel complex to traffic to the cell surface. An additional anterograde trafficking signal is present in the COOH terminus of SUR1, and deletion of as few as seven amino acids from the COOH terminus of SUR1 markedly reduces surface expression of $K_{ATP}$ channels (54). For such mutations, manipulations that allow correction of the trafficking defects might be of therapeutic value for the disease. Cartier et al. (11) found that mutation of the RKR sequence to AAA leads to partial expression of SUR1[ΔF1388] mutant channels (Fig. 2A). In this particular case, the now expressed channels also show the other common HI defect and are still MgADP insensitive (Fig. 2B), such that even if the trafficking defect were corrected, the mutant channel would remain physiologically nonfunctional. Partridge et al. (47) found that whereas lowering the temperature did not improve surface expression of SUR1[R1394H], treatment with diazoxide did, an effect that could be blocked by simultaneous treatment with glibenclamide. It may be of potential therapeutic importance to determine whether diazoxide treatment affects the expression efficiency of wild-type channels and other trafficking mutant channels.

**Epidemiology of HI: Clinical Correlations Between Mutation and Disease**

Because $K_{ATP}$ channels are formed as octamers, consisting of four Kir6.2 and four SUR1 subunits, the degree of dominance of any disease mutations is expected to correlate with the degree of impairment of insulin release and severity of the HI disease that result. In heterozygous conditions, heteromeric channels will predominate, and partial reductions of channel activity will be expected. In cases where isolated β-cells have been examined from HI patients, complete inhibition of $K_{ATP}$ channel activity leading to constitutive membrane depolarization, activation of voltage-gated Ca$^{2+}$ channels, and inappropriate exocytosis of insulin, regardless of the blood glucose levels, have been observed (1, 29). In future studies, detailed analysis of genotype-phenotype correlations for the wide range of different HI mutations in $K_{ATP}$ channel genes is likely to reveal a range of phenotypic outcomes, given the low allelic frequencies of single mutations and the fact that most patients with HI-associated mutations are compound heterozygotes or have only a single identified mutation. Patients from genetically isolated populations (like Ashkenazi Jews or Finns) offer controlled cohorts for study of genotype-phenotype correlations, and a database integrating the clinical, molecular genetic, histopathological, and electrophysiological data of all European patients is under development and will enable more detailed correlation studies in the future (http://umd2.necker.fr:2007/).

One piece of evidence for genetic heterogeneity of HI is that specific mutations in $K_{ATP}$ channel genes may explain HI to varying degrees in different populations. For example, in the Japanese population, SUR1 mutations account for only ~20% of HI cases, whereas in Ashkenazi Jews, two single mutations account for 90% of all cases (44, 64). The deletion of the codon for F1388 (AF1388) was found to associate with 20% of the Ashkenazi Jewish HI-associated chromosomes (44). Homozygous AF1388 was detected in only two patients, who both had a severe HI. The splice site mutation 3992–9g→a, which has been detected in 70% of Ashkenazi Jewish HI chromosomes, leads to variable phenotypic expression of the disease (67): most patients who are homozygous for this mutation have a severe drug-resistant form of the disease, but others have mild HI and are clinically unaffected. The clinical heterogeneity may be due to the effects of other genes or exogenous factors that modify the phenotype, and individuals with different phenotypes may express variable proportions of normal protein (44).

Two different founder mutations in the SUR1 gene are associated with >50% of all HI cases in the Finnish population, and each mutation is geographically clustered in distinct regions of the country (Fig. 3A). The recessively inherited missense mutation V187D, located in a transmembrane domain of SUR1, leads to severe early-onset HI (46). This disease is geographically clustered in Central Finland (Fig. 3A), where the incidence is as high as 1:3,200 births. Interestingly, the disease phenotype is almost as severe in patients homozygous or heterozygous for the mutation; even a single copy of the V187D mutation seems to lead to a severe drug-unresponsive form of HI in compound heterozygotes. However, carriers of this mutation (parents, siblings) are asymptomatic and have normal insulin secretion, normal tissue sensitivity to insulin, and no inappropriate insulin secretion during hypoglycemia (26). Functional studies (intact cell recordings, cell-free inside-out patches) of β-cells isolated from an HI patient homozygous for the V187D mutation, as well as the results of recombinant $K_{ATP}$ channel experiments, are consistent with the phenotype and show that mutation SUR1[V187D] leads to a loss of functional $K_{ATP}$ channels that are not activated by diazoxide or somatostatin.

The dominantly inherited mutation SUR1[E1506K] (Fig. 3B) associates with a different phenotype (25). All patients have a mild form of HI that can usually be managed by long-term diazoxide treatment. This clinical finding is in agreement with the results of coexpression studies of recombinant wild-type (wt)-Kir6.2 and SUR1[E1506K]. Mutant channels are insensitive to metabolic inhibition, but a partial response to diazoxide is retained. Despite the dominant nature of SUR1[E1506K] in causing the disease, it does not exert a completely dominant negative effect when expressed...
together with the wild-type gene in Xenopus oocytes. Studies of glucose homeostasis in carriers of the SUR1[E1506K] mutation have indicated that this mutation leads to insulin deficiency and to development of diabetes mellitus in later life (25).

In roughly one-third of all cases, focal adenomatous hyperplasia (focal HI), with somatic loss of maternal alleles in the imprinted chromosomal region 11p15 along with paternally inherited KATP channel mutations, leads to the HI phenotype (13, 17, 21, 73). The focal form of HI may be genetically more homogenous than diffuse HI, as it is linked to KATP channel gene mutations in almost two-thirds of cases (17). The lost maternal region includes the nonimprinted SUR1 and Kir6.2 genes, the maternally imprinted tumor suppressor genes H19 and P57KIP2, and the paternally imprinted insulin-like growth factor II, which plays a central role in pancreatic tumorigenesis. The imbalance between the tumor suppressor genes and IGF-II leads to focal hyperplasia of β-cells, whereas the rest of the pancreas presents a normal histology (53). Despite the differential genetic etiologies, the clinical presentation of focal and diffuse forms of HI is similar, and these two conditions can be distinguished only by selective venous catheterization and perioperative microscopic examination of frozen sections (52). In the case of focal HI, patients can be treated by a partial resection of the focal lesion, whereas in the diffuse form of the disease, subtotal pancreatectomy is often necessary (33, 62).

Mutations in genes encoding the glucokinase (GK) and glutamate dehydrogenase (GDH) enzymes are rare causes of HI. GK controls the rate-limiting step of β-cell glucose metabolism and is therefore critical in glucose-mediated insulin secretion (35). Glaser et al. (20) described a unique autosomal dominant missense mutation V455M in the GK gene that leads to increased glucose affinity. The mutation was detected in a single family with five affected individuals in three generations. All patients responded well to diazoxide treatment, consistent with the ability to bypass the defect and activate KATP channels directly. Interestingly, insulin-deficient diabetes also developed later in life in the oldest family member, suggesting that the mutation may cause gradual β-cell failure.

Hyperinsulinism-hyperammonemia (HIHA) is a syndrome caused by dominantly inherited activating mutations in the GDH gene that simultaneously increase the release of insulin by pancreatic β-cells and impair the detoxification of ammonia in the liver (62). Most mutations are located in the allosteric regulatory domain of the enzyme, but recent studies have described mutations outside this region (41, 49, 61). Hypoglycemia in patients with HIHA is generally less severe than in patients with mutations in KATP channel genes. The clinical manifestations of HIHA include normal birth weight, late onset of hypoglycemia, diazoxide responsiveness, and protein-sensitive hypoglycemia (62).

**ANIMAL MODELS OF HI**

There has been variable success at generating mouse models for HI by manipulation of the SUR1 or Kir6.2 genes (69, 70). Miki et al. (40) first generated transgenic mice expressing a dominant-negative mutant of
Kir6.2 (Kir6.2\{G132S\}) in β-cells under control of the insulin promoter. The mutation alters the structure of the K⁺ selectivity filter, rendering the channels either nonfunctional or possibly slightly Na⁺ permeable (42). KₐTP currents are significantly reduced in isolated β-cells, and both resting membrane potential and basal intracellular calcium concentration ([Ca²⁺]ᵢ) are consequently significantly higher than those of control mice. Neonatal transgenic mice exhibit relatively high levels of serum insulin despite hypoglycemia, resembling HI in humans, but the transgenic mice rapidly develop hyperglycemia with reduced glucose-induced insulin secretion. Histological analysis reveals abnormal architecture of the islets of transgenic mice, with enhanced apoptosis and a marked decrease in the number of β-cells in adult mice.

Miki et al. (39) subsequently knocked out the Kir6.2 gene by homologous recombination to generate mice completely lacking KₐTP channels in β-cells of homozygous (Kir6.2\{−/−\}) mice, and again [Ca²⁺]ᵢ and membrane potentials were elevated. The mice also show a transient hypoglycemia as neonates similar to that of Kir6.2\{G132S\} transgenic mice. Again, there is no glucose-dependent insulin secretion, and older animals are glucose intolerant. Surprisingly, glucose tolerance is apparently normal in young mice, and insulin tolerance is actually enhanced, possibly a consequence of reduced KₐTP channel activity in skeletal muscle cells. Seghers et al. (50) generated SUR1 knockout mice. These animals also completely lack KₐTP channels in β-cells and might reasonably be expected to provide the most appropriate HI model. First-phase insulin release is almost completely abolished, as in Kir6.2\{−/−\} mice, and a second-phase insulin release is reduced compared with wild-type animals. Blood glucose levels are normal in adult animals; however, consistent with an inability to “turn off” insulin release by KₐTP channel activation, fasted SUR1\{−/−\} mice did show statistically significant hypoglycemia relative to control SUR1\{+/+\} mice (50). Nevertheless, frank hypoglycemia and abnormally elevated insulin-to-glucose ratios were really observed only in the 1st day of life for SUR1\{−/−\} mice, and by day 5, the situation had reversed to a hyperglycemic phenotype. Clearly, certain incretins can bypass the KₐTP channel to more directly induce insulin secretion, and even though glucose-induced insulin secretion is greatly reduced or abolished in Kir6.2\{−/−\} and SUR1\{−/−\} mice, in each case there is minimal impairment of glucose tolerance, and blood glucose is normal in young animals. Insulin responses to either intraperitoneal glucose loading or to meal ingestion are observed in Kir6.2\{−/−\} mice (51), suggesting that mixed meal-induced insulin secretion due to potentiating effects of incretins is retained (51).

Thus these various knockout animals reiterate the expected cellular phenotypes (i.e., abolition of KₐTP channels and elevated [Ca²⁺]ᵢ) that are expected to underlie HI. However, in no case was persistent hyperinsulinemia observed, and rapid reversal of any transient neonatal hypoglycemia resulted in a hyperglycemic, essentially diabetic, phenotype. The reasons for the lack of correlation between the mouse phenotypes with HI in humans are not entirely clear. Although temporally uncorrelated, there is evidence that HI patients may cross over to a diabetic phenotype in later life, although this has been attributed to the near-total pancreatectomy that is acutely required to treat the neonatal symptoms (31). However, as we have mentioned, there are recent studies indicating that nonsurgically treated HI patients may become diabetic in later life (23, 25). Conceivably, β-cell death (as observed in mice expressing Kir6.2 dominant-negative Kir6.2 constructs (40)), coupled with a decreased glucose-dependent insulin release (as demonstrated in both knockout mice (39, 50) and in SUR1\{−/−\} HI patients (23)) may underlie a later onset of diabetes. In this context, it should be noted that, because KₐTP-dependent insulin secretion requires dynamic variations of KₐTP channel activity, dynamic control can be abolished either by abolition of channel activity or by raising channel activity to a constant, unregulated level. Mutations that make channel activity high may therefore be expected to cause a primary hypoinsulinemic diabetes. In accord with this prediction, mice expressing a gain-of-function Kir6.2\{ΔN30\} transgene that reduces ATP sensitivity of expressed channels are dramatically hypoglycemic and hyperinsulinemic as neonates, typically dying within 1 wk of birth (30). It remains an open question whether any gain-of-function KₐTP channel mutations underlie a MODY (Maturity Onset Diabetes of the Young) or other non-type I diabetic phenotype in humans.

PERSPECTIVES

The last five or six years have seen dramatic elucidation of the molecular basis of KₐTP channel function and the role of KₐTP channels in insulin secretion, highlighted by the critical causative role of mutations of the channel subunits in the HI disease. Recombinant expression experiments have demonstrated the underlying molecular defects, and as patients are genotyped, the correlation of clinical phenotype with underlying defect is being pursued. It is important to note that the multimeric nature of the KₐTP channel will generate multiple channel phenotypes and consequences in a heterozygous condition, and this complexity will be compounded by the influences of other genes on both channel expression and the cellular metabolism and electrical substrates. Future phenotypic studies of KₐTP channel dysfunction in HI patients and in animal models should have implications both for understanding the pathophysiological mechanisms and potential treatments of the HI disease and also for mechanisms of impaired insulin secretion in the far more common type II diabetes.

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