Perspectives in Diabetes

Diabetes and Insulin Secretion

The ATP-Sensitive K^+ Channel (K_{ATP}) Connection

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The ATP-sensitive K^+ channel (K_{ATP} channel) senses metabolic changes in the pancreatic β-cell, thereby coupling metabolism to electrical activity and ultimately to insulin secretion. When K_{ATP} channels open, β-cells hyperpolarize and insulin secretion is suppressed. The prediction that K_{ATP} channel "overactivity" should cause a diabetic state due to undersecretion of insulin has been dramatically borne out by recent genetic studies implicating "activating" mutations in the Kir6.2 subunit of K_{ATP} channel as causal in human diabetes. This article summarizes the emerging picture of K_{ATP} channel as a major cause of neonatal diabetes and of a polymorphism in K_{ATP} channel (E23K) as a type 2 diabetes risk factor. The degree of K_{ATP} channel "overactivity" correlates with the severity of the diabetic phenotype. At one end of the spectrum, polymorphisms that result in a modest increase in K_{ATP} channel activity represent a risk factor for development of late-onset diabetes. At the other end, severe "activating" mutations underlie syndromic neonatal diabetes, with multiple organ involvement and complete failure of glucose-dependent insulin secretion, reflecting K_{ATP} channel "overactivity" in both pancreatic and extrapancreatic tissues. *Diabetes* 54:3065–3072, 2005

In the pancreatic β-cell, the ATP-sensitive K^+ channel (K_{ATP} channel) plays an essential role in coupling membrane excitability with glucose-stimulated insulin secretion (GSIS) (1). An increase in glucose metabolism leads to elevated intracellular [ATP]/[ADP] ratio, closure of K_{ATP} channels, and membrane depolarization. Consequent activation of voltage-dependent Ca^{2+} channels causes a rise in $[Ca^{2+}]_i$, which stimulates insulin release (Fig. 1). Conversely, a decrease in the metabolic signal is predicted to open K_{ATP} channels and suppress the electrical trigger of insulin secretion. Sulfonylurea drugs promote, and diazoxide suppresses, insulin secretion by binding to the regulatory sulfonylurea receptor-1 (SUR1) subunit and inhibiting, or activating, K_{ATP} channel current, respectively (2). The electrical pathway is modulated by K_{ATP} channel–independent mechanisms; nutrient metabolites and incretins affect secretion at various stages downstream of K_{ATP} channel (3,4), but the drug effects underscore the central role of K_{ATP} channel–dependent regulation.

Alterations in the metabolic signal, in the sensitivity of K_{ATP} channel to metabolites, or in the number of active K_{ATP} channels, could each disrupt electrical signaling in the β-cell and alter insulin release. In support of this prediction, earlier studies implicated reduced or absent K_{ATP} channel activity in the β-cell as causal in congenital hyperinsulinism (CHI) in humans (5). CHI is a rare, mostly recessive, disorder characterized by constitutive insulin secretion despite low blood glucose. If left untreated, severe mental retardation and death may result. Mutations in K_{ATP} channel that reduce channel expression, decrease stimulation of the channel by MgADP, or abolish channel activity account for a majority of all CHI mutations (6–9). Conversely, mutations that result in "overactive" channels should decrease membrane excitability and impair glucose sensing by the β-cell. In this scenario, insulin secretion will be reduced and a diabetic phenotype is predicted. A clear picture is now emerging from both animal and human studies that such K_{ATP} channel mutations can indeed cause diabetes. The first part of this review will detail the rapidly emerging clinical evidence for involvement of K_{ATP} channel mutations in neonatal and type 2 diabetes and the cellular basis of the disease. The second part will consider the structure-function relationships of the K_{ATP} channel and molecular mechanisms that underlie diabetes in which mutations in K_{ATP} are causal.

PART 1: B-CELL K_{ATP} CHANNEL AND DIABETES: THE EMERGING GENETIC AND CLINICAL PICTURE

Mutations in Kir6.2 underlie neonatal diabetes. Given the above paradigm, any gain of K_{ATP} channel function is expected to suppress GSIS. This prediction was originally confirmed by the striking neonatal diabetic phenotype of two different genetic models: *1*) mice with targeted disruption of the pancreatic β-cell glucokinase gene (10) and *2*) transgenic mice expressing β-cell K_{ATP} channels with decreased sensitivity to inhibitory ATP (i.e., "overactive" K_{ATP} channel) (11). In each case, acute neonatal hyperglycemia together with ketoacidosis, leading to death within a few days, was observed. In the latter model (Fig. 2*A*), blood insulin is at or below the level of detection but insulin is clearly present in the pancreas (11). In each case, overactive K_{ATP} channel activity, with a failure to switch on insulin secretion, is the logical underlying mechanism; this is due to altered metabolic signal in the former (12) and insensitivity to the normal metabolic signal in the latter (11).

The obvious prediction of these mouse models is that genetically induced ATP insensitivity of β-cell K_{ATP} chan-

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CHI, congenital hyperinsulinism; FOXP3, forkhead box P3; GSIS, glucose-stimulated insulin secretion; IPF-1, insulin promoter factor 1; K_{ATP} channel, ATP-sensitive K^+ channel; PIP_2, phosphatidylinositol-4,5-bisphosphate; SUR1, sulfonylurea receptor-1.

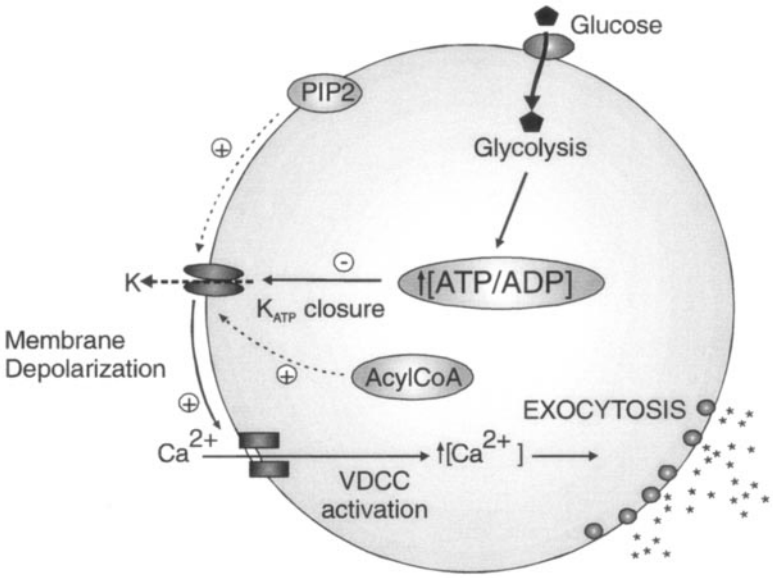

FIG. 1. The role of pancreatic K_{ATP} channel in insulin secretion. Elevated blood glucose increases glucose metabolism in the β-cell and elevates [ATP]/[ADP]$_i$. This metabolic signal closes K_{ATP} channels, causing depolarization, activation of voltage-dependent Ca^{2+} channels, Ca^{2+} entry, and insulin exocytosis. Various additional effectors, including PIP_2 and acyl CoAs, act to modulate ATP sensitivity of the channel and can thereby affect the coupling of metabolism to secretion.

nels could underlie impaired insulin release and neonatal diabetes in humans (13,14). Rare in occurrence (1:400,000 births), neonatal diabetes is usually diagnosed within the first 3 months of life and relies on insulin administration to treat the hyperglycemia (15). In transient neonatal diabetes, which is milder, hyperglycemia usually resolves within 18 months, whereas the permanent form requires insulin treatment for life. Until recently, the cause of the majority of permanent neonatal diabetes cases has remained unknown. Homozygous and compound heterozygous mutations in glucokinase, the rate-limiting enzyme of glucose metabolism in islet cells, cause isolated permanent neonatal diabetes and account for a minority of cases (at least six families reported to date) (16,17). Similarly, compound heterozygous or homozygous mutations in insulin promoter factor 1 (IPF-1), an essential transcriptional regulator of pancreatic development, underlie a fewer number of cases (18,19). Other rare forms of permanent neonatal diabetes are associated with multiple deficiencies. These include Wolcott-Rallison syndrome, characterized by infancy-onset diabetes along with growth and mental retardation and caused by mutations in EIF2AK3, a regulator of protein synthesis (20,21). In addition, defects of forkhead box P3 (FOXP3) underlie IPEX (immunodysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome, which also includes neonatal-onset diabetes (22). While defects in glucokinase and K_{ATP} channel are predicted to impair glucose sensing of the β-cell, leading to suppressed insulin release, mutations in IPF-1 and FOXP3 underlie decreased β-cell mass; IPF-1 through impaired pancreatic development and FOXP3 through autoimmune destruction of pancreatic islets (23,24). In contrast to permanent neonatal diabetes, a majority of transient neonatal diabetes cases are attributable to paternal imprinting at chromosome 6q24 (25). Two candidate genes have been identified: *ZAC* (zinc finger protein that regulates apoptosis and cell-cycle arrest) and HYAMI (hydatidiform mole-associated and imprinted transcript), an untranslated mRNA of unknown function.

Recent genetic studies demonstrate that heterozygous mutations in *KCNJ11*, encoding the Kir6.2 subunit of the K_{ATP} channel, underlie neonatal diabetes in humans, accounting for both permanent neonatal diabetes (26–31), in which type 1 autoantibodies are absent, and relapsing transient neonatal diabetes, in which chromosome 6 abnormalities were excluded (32,33). Both de novo appearance of Kir6.2 mutations and familial transmission have been reported (26–31). Significantly, in all examined families, neonatal diabetes was observed only in individuals carrying the Kir6.2 mutations and not in other family members. Interestingly, in a subgroup of patients carrying Kir6.2 mutations, permanent neonatal diabetes is part of a larger syndrome that often includes marked developmental delay in motor intellectual and social skills, muscle weakness, dysmorphic features, and epilepsy (27,29,30). Despite normal-sized cortex and cerebellum, a majority of patients with syndromic permanent neonatal diabetes also display language and social development that is delayed from 5 to 48 months (27). There appears to be no correlation between the age of diagnosis and the severity of the permanent neonatal diabetes (syndromic versus nonsyndromic). As Kir6.2 represents the pore-forming subunit of K_{ATP} channels in skeletal muscle and in neurons throughout the brain (34,35), differentially overactive K_{ATP} channels in extrapancreatic tissue can potentially account for neurological disorders associated with this subgroup of patients through as-yet-to-be-defined mechanisms.

Neonatal diabetic subjects with Kir6.2 mutations demonstrate varying levels of C-peptide (27,31–33,36) consistent with a varying degree of β-cell dysfunction and likely accounting for the variable hyperglycemia often observed with neonatal diabetes. Consistent with a defect at the level of K_{ATP} channel, affected patients carrying R201 mutations did not secrete insulin in response to glucose or glucagon but did in response to sulfonylurea (tolbutamide), albeit at subnormal levels (Fig. 2*B*) (27,30). Importantly, several patients have now been weaned from insulin onto glibenclamide therapy, and at 1- to 6-month follow-ups, blood glucose has been well controlled without insulin supplement (31,36). In many cases, however, the oral dosage of sulfonylureas significantly exceeds (by several-fold) the doses commonly used to treat type 2 diabetes (36,37). Consistent with this clinical observation, the neonatal diabetes–causing mutations in Kir6.2 are frequently associated with a concomitant decrease in sensitivity of the K_{ATP} channel to sulfonylureas (27,38,39), which may underlie the increased therapeutic dosages.

As additional mutations are uncovered, it is becoming

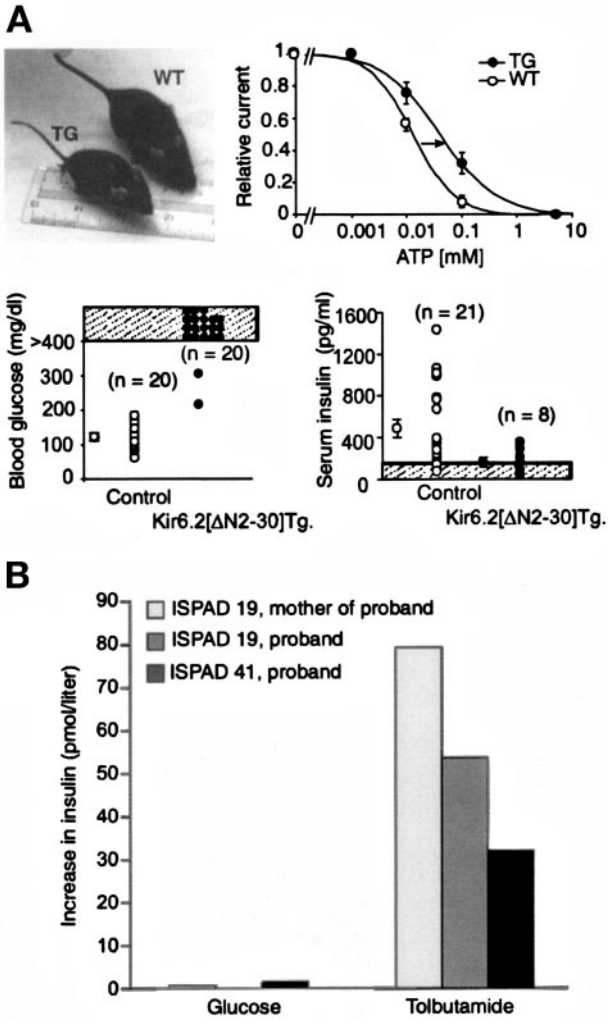

FIG. 2. K_{ATP} channel–dependent diabetes in mouse and humans. *A*: *Upper left*: In Kir6.2[ΔN30]-expressing transgenic (TG) mice, only 3 of ~150 F1 transgenic mice survived to weaning (11). Those that did were approximately two-thirds the body weight of wild-type (WT) littermates. *Upper right*: Steady-state dependence of K_{ATP} channel current on [ATP] was shifted approximately fivefold to higher [ATP] in membrane patches from transgenic β-cells. *Lower*: Blood glucose (*left*) and serum insulin (*right*) levels were at or outside the level of detection in 2- to 4-day neonatal transgenic mice (11). *B*: Peak serum insulin levels compared with baseline in three permanent neonatal diabetes patients carrying R201 mutations in Kir6.2. Glucose, administered intravenously at 0.3 g/kg, failed to elicit an insulin response, but significant insulin was released in response to tolbutamide (at 3 mg/kg). ISPAD, International Society for Pediatric and Adolescent Diabetes. Republished from Gloyn et al. (27) with permission.

clear that the temporal presentation of the disease can be quite variable. One patient was diagnosed at only 26 weeks of age (27) and another at 5 years (32). Within a single pedigree, one Kir6.2 mutation (C42R) is shown to underlie transient neonatal diabetes, childhood-onset diabetes, as well an apparently type 2 diabetes, all in different carriers (33). Such late presentation suggests that the disease may become apparent at much later ages than typically ascribed, consistent with the notion that the mildest forms of the disease may not manifest until adulthood. This raises the additional possibility that if the disease manifests postnatally, it may be misdiagnosed as type 1 diabetes. So far, however, screening of children diagnosed with type 1 diabetes before 2 years of age, and lacking predisposing HLA genotypes, has failed to demonstrate a significant number of *KCNJ11* mutations (28).

Polymorphisms of Kir6.2 predispose to adult-onset diabetes. Numerous studies have now examined the association of K_{ATP} channel polymorphisms with late-onset type 2 diabetes (40–44). By suppressing excitability, K_{ATP} channel polymorphisms that increase channel activity could, in combination with other environmental and genetic factors, contribute to chronically impaired β-cell function. In the face of insulin resistance, this is expected to exacerbate the hyperglycemic state. Although results from initial studies are conflicting (45,46), large-scale association studies and meta-analyses have now identified the E23K polymorphism in *KCNJ11* as a slight, but significant, risk factor in the complex development of type 2 diabetes (42,44,47–50). However, given the high allelic frequency of E23K in the general population (frequency of heterozygous EK genotype = 47%; homozygous KK genotype = 12%), the polymorphism is likely to represent a large population-attributable risk (41,48,51,52). Importantly, a recent haplotype analysis of the Kir6.2/SUR1 gene region has demonstrated a strong allelic association of E23K in Kir6.2 with a polymorphism in SUR1 (A1369S), raising the possibility that E23K alone may not entirely account for the reported association with type 2 diabetes (49).

Cellular basis of K_{ATP} channel diabetes. Recombinant expression of mutant channels indicates that both the type 2–associated polymorphism (E23K) and neonatal diabetes–associated Kir6.2 mutations result in reduced sensitivity to intracellular ATP, either by reducing ATP affinity per se or indirectly via an increase in the intrinsic open-state stability (27,38,39,51,53) (see below). At the cellular level, an important question is: Just how much change in ATP sensitivity is necessary to cause significant impairment of insulin secretion? The diabetic phenotype of transgenic mice expressing "overactive" K_{ATP} channels in β-cells predicted the correlate disease in humans and is a potentially relevant model (11). These mice express Kir6.2 subunits with truncated NH_2-termini, which causes a 10-fold reduction of ATP sensitivity in heterologously expressed channels. Transgenic F1 mice from four of five founder lines expressing the truncated channels were severely hyperglycemic, and hypoinsulinemic, and died as neonates by day 5, most likely from acute ketoacidosis (Fig. 2*A*). Electrophysiology confirmed functional expression of K_{ATP} channels with reduced ATP sensitivity, but only approximately fourfold relative to wild type.

Heterologously expressed Kir.2[E23K]-SUR1 channels exhibit even more modest 2- and 1.5-fold reductions in ATP sensitivity for homozygous (Kir6.2[E23K]) and heterozygous (Kir6.2[E23K] + Kir6.2wt) channels, respectively (51 and J.C.K., unpublished observations: $K_{1/2\ [ATP]}$ for Kir6.2wt = 10.7 ± 1.9 μmol/l, Kir6.2[E23K] = 17.6 ± 0.9 μmol/l [expressed in COSm6 cells]), as well as enhanced MgADP stimulation (51–54). E23K is in linkage disequilibrium with another Kir6.2 polymorphism, I337V, which itself has no reported effect on channel activity (51,53). Another study reported no reduction of ATP sensitivity of E23K/I337V channels, but instead showed enhanced stimulatory effects of palmitoyl-CoA on E23K/I337V mutant Kir6.2 channels (55). As discussed below, a small increase in intrinsic open-state stability can underlie a decrease in apparent ATP sensitivity, as well as an increase in sensitivity to activator molecules (51–54,56). It seems likely that increased palmitoyl-CoA sensitivity in the latter study, as well as increased MgADP sensitivity and reduced ATP sensitivity in the previous study, all reflect just such an

increase. In all cases, the net effect will be reduced glucose sensing by the β-cell, and, in support, a small effect of the E23K variant on insulin release was observed during intravenous and oral glucose tolerance tests (43,57,58).

E23K also has a strong allelic association with a SUR1 polymorphism (A1369S), raising the possibility that the SUR1 variant may influence, or account for, altered channel activity (49). The effect of the A1369S polymorphism on channel activity is not known (51,55), but the possibility should be acknowledged that alone or in combination with E23K, it contributes to altered ATP sensitivity. (In our unpublished studies of E23K, reconstituted K_{ATP} channels carried both the I337V and A1369S polymorphisms.)

What of the ATP sensitivity of different neonatal diabetes mutants? Gloyn et al. (27) initially showed an ~35-fold loss of ATP sensitivity for homozygous R201H mutant channels, but almost no shift in a 1:1 mixture of wild-type and R201H mutant subunits, expected to recapitulate the heterozygous state of the disease. Assuming a 1:1 expression and random assembly, 16 different subunit arrangements are formally possible, making the analysis of mixed expression very complex (51,53,59,60). However, as acknowledged, 1 of 16 of the expressed channels are expected to be pure mutant, and this alone could give rise to significant currents at physiological [ATP]/[ADP] ratios. Other mutations (Q52R, I296L I182V, V59G, V59M, Y330C, and F333I) have now been analyzed, demonstrating shifts in ATP sensitivity of up to ~1,000-fold for homozygous V59G and I296L mutant channels (38,39). Again, much lesser shifts were observed in heterozygous expression, but, without analyzing each subunit combination separately, it remains speculative as to exactly what channel activity can be expected in vivo.

Nonelectrical consequences of altered K_{ATP} channel activity. At this juncture, we cannot preclude nonelectrical secondary mechanisms underlying K_{ATP} channel–induced diabetes. Mice with overactive β-cell K_{ATP} channels are profoundly diabetic within a few days of birth (11). Morphologically, the size, distribution, and architecture of the islets are unperturbed at the earliest stages of diabetes (days 1–3), but collapse of islet architecture, with diffuse distribution of the α- and β-cells throughout the pancreas, was observed at later stages (after day 3). A similar mechanistic progression may occur in K_{ATP} channel–induced permanent neonatal diabetes and may underlie some of the reduced sulfonylurea-sensitive insulin release (27). Moreover, a recent transgenic study overexpressing the transient neonatal diabetes locus (6q24) implicated fluctuations in β-cell mass and insulin content in the progression of transient neonatal diabetes from the neonatal diabetic phase into remission and ultimately to late-onset diabetes (61). If a similar pathophysiology occurs in transient neonatal diabetes patients carrying Kir6.2 mutations, this would be consistent with secondary, nonelectrical consequences of altered β-cell K_{ATP} channel activity. Secondary complications do occur in the converse disease progression resulting from reduced K_{ATP} channel density. Transgenic mice lacking K_{ATP} channels in ~70% of β-cells, due to β-cell expression of dominant-negative Kir6.2 transgene, hypersecrete throughout as adults (14), but mice completely lacking K_{ATP} channels are reportedly hyperinsulinemic as neonates and then progress to reduced GSIS and glucose intolerance as adults (62–64). Importantly, when exposed to a high-fat diet, both Kir6.2$^{-/-}$ mice and Kir6.2[AAA] transgenic mice progress rapidly to severely undersecreting diabetes (65). While these hyperexcitable mice, with reduced K_{ATP} channel activity, thus have a very different response to those with overactive K_{ATP} channels, they do suggest that profound nonelectrical consequences can follow an initial electrical disturbance.

In addition to Kir6.2 defects, loss or reduction of K_{ATP} channel activity can also occur due to loss-of-function mutations of the regulatory SUR1 subunit; such mutations are the most frequent cause of CHI (5). Clinical data indicate that CHI patients carrying SUR1 mutations can also progress to glucose intolerance and, in some cases, overt diabetes (66).

Finally, non–β-cell mechanisms must be considered. Kir6.2 is the pore-forming subunit of α-cell, muscular, and neuronal K_{ATP} channels (34). In skeletal muscle, or in neurons, overactive K_{ATP} channel could potentially underlie the muscle weakness reported in syndromic permanent neonatal diabetes (27,29). Glucose uptake by skeletal muscle and adipose may also be dependent on K_{ATP} channel activity (67), and overactivity in syndromic permanent neonatal diabetes could compromise this function. In neurons, metabolic sensing is altered with loss of K_{ATP} channels (35,68). Again, the unknown consequences of K_{ATP} channel overactivity could underlie developmental defects that are also observed in syndromic permanent neonatal diabetes (27,29). Complete understanding of the mechanisms by which Kir6.2 mutations cause neonatal diabetes will require mechanistic molecular analysis of disease-causing mutations (32,33,38,39), generation of transgenic mouse models of neonatal diabetes (11), and analysis of virally infected β-cells or insulinoma cell lines expressing altered K_{ATP} channel.

PART 2: K_{ATP} CHANNEL–DEPENDENT DIABETES: THE MOLECULAR DEFECTS

The K_{ATP} channel: structure and function. Structurally, the pancreatic K_{ATP} channel consists of two unrelated subunits: a sulfonylurea receptor (the SUR1 isoform) that is a member of the ABC transporter family and a potassium channel subunit (Kir6.2) that forms the central ion-conducting pathway (Fig. 3*A*). The mature K_{ATP} channel exists as an octamer of Kir6.2 and SUR1 subunits in a 4:4 stoichiometry (Fig. 3*B*) (59,69,70). The signature ATP inhibition of the channel results from binding to the pore-forming Kir6.2 subunit (71–76). Importantly, the inhibitory concentration of ATP causing half-maximal channel inhibition is in the micromolar range ($K_{1/2[ATP]}$ ~10 μmol/l for native K_{ATP} channel) (77), yet cytosolic [ATP] is in the millimolar range (1–5 mmol/l) and changes little in the presence of high glucose (78). This observation implicates MgADP, rather than ATP, as the primary physiological regulator of channel activity. Through interaction with SUR1, MgADP "stimulates" channel activity by countering ATP inhibition (8,79–83). SUR1 mutations that abolish MgADP action, but do not alter ATP sensitivity, also abolish channel activity in vivo and underlie CHI (8,84). It should be pointed out, however, that despite the essential regulatory role of MgADP, ATP sensitivity of the channel will still be critical in determining the magnitude of K_{ATP} channel current. For a given [MgADP], K_{ATP} channel mutations that decrease ATP sensitivity will enhance absolute currents in the physiologic range of ATP, and the net effect of enhanced K_{ATP} channel current is a decrease in β-cell excitability.

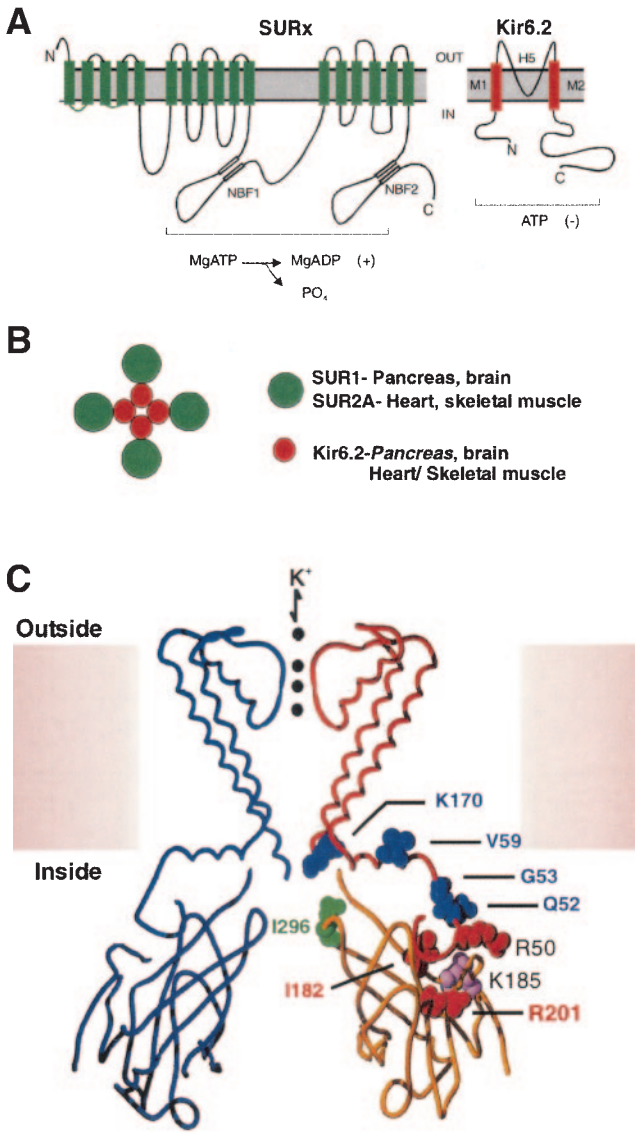

FIG. 3. Molecular basis of K_{ATP} channel–induced diabetes. *A*: Membrane topology of SUR1x and Kir6.2 subunits of the K_{ATP} channel. ATP inhibits K_{ATP} channels by binding to the Kir6.2 subunit. Nucleotide hydrolysis at the SUR1 nucleotide-binding folds (NBFs), or MgADP binding, is thought to counteract the inhibitory effect of ATP. *B*: Predicted octameric structure of K_{ATP} channel. The functional channel consists of SUR1 or SUR2A and Kir6.2 subunits in a 1:1 stoichiometry. As shown from the top view, four Kir6.2 subunits surround and form a central pore. *C*: Ribbon diagram of two opposing cytoplasmic domains and two opposing transmembrane domains of Kir6.2, modeled on the crystal structure of KirBac1.1 (86). Many Kir6.2 residues mutated in human neonatal diabetes are indicated, together with K185, which is an additional residue that is predicted to line the ATP-binding cavity (see text for complete list of Kir6.2 residues mutated in neonatal diabetes). Residues are classified as those putatively involved in ATP binding (red and pink) or in regulation of open-state stability (blue and green). The type 2 diabetes–associated polymorphism E23K is located in the far NH_2-terminus, for which a predicted structural location is unavailable.

Molecular mechanisms of K_{ATP} channel–induced neonatal diabetes. Multiple permanent neonatal diabetes mutations have now been identified in Kir6.2 (Fig. 3*C*). To date, the most frequently mutated residues are R201 and V59; heterozygous mutations of these residues have been detected in multiple probands (27,29,30). Y330C accounted for three cases of permanent neonatal diabetes (28,30), whereas K170 substitutions accounted for two permanent neonatal diabetes cases (K170N and K170R) (29). Q52R, I296L, R50P, F33I, and E322K have all been found in one proband each (28,29,31). Kir6.2 mutations causing transient neonatal diabetes include G53, I182, and C42 (32,33).

These mutations could reduce ATP sensitivity *1*) directly by decreasing the affinity of the ATP-binding pocket for the nucleotide, *2*) indirectly by an increase in the intrinsic stability of the open state of the channel, or *3*) indirectly by an increased sensitivity to the counteractivation by MgADP or by phosphatidylinositol-4,5-bisphosphate (PIP_2) or other phospholipids. Consistent with a direct effect on binding, R50, I182, Y330, F333, and R201 have all previously been implicated as putative ATP-binding residues in Kir6.2 (27,74,85). Modeling of the COOH-terminus of Kir6.2, based on homology with the crystal structure of the related Kir3.1, predicts that I182 resides in a hydrophobic pocket that coordinates the adenine ring of ATP (75), while positively charged R201 and R50 are predicted to interact electrostatically with the phosphate tail of ATP (75,85,86). Both Y330 and F333 also predicted to lie close to the phosphate tail in the binding pocket (87).

Fully consistent with the above structural model of the ATP-binding pocket, functional characterization now demonstrates a significant decrease in the ATP sensitivities of these mutants (27,32,38,39). In the case of I182V, R201C, R201H, and F333I, these mutations do not alter gating of the channel and are, therefore, likely to directly alter ATP affinity at the binding pocket. A few additional residues in the NH_2- and COOH-terminus are also likely to be involved in ATP binding (71,73,75,76,88,89), and we speculate that substitutions at these residues would also be disease causing.

With respect to indirect channel mechanisms, mutations that alter channel gating in the absence of ATP can have profound effects on apparent ATP sensitivity (73,76,90). Such mutations are found throughout the Kir6.2 subunit. In some cases, these "open-state stability" mutations are located in transmembrane segments, a significant distance from the putative ATP-binding domain (86,91). Many, including E23K, stabilize the open state of the channel, thereby increasing the channel opening independently of ATP and indirectly reducing the apparent affinity for ATP (51,54). The reported loss of ATP sensitivity coupled with high open probability in the Q52R, C42R, Y330C, I1296L, and V59G mutations suggests such a mechanism in these disease mutations (38,39). Consistent with the functional interpretation, Q52 and V59 are located within the predicted slide helix region of Kir6.2 and may regulate channel gating by coupling the ATP-binding site physically to the gating region (75,86). The second transmembrane helices in each of the Kir6.2 subunits converge at the so-called "bundle crossing" and may form the ligand-controlled gate (Fig. 3*C*) (86,91). An effect on the gate region of the channel might then explain the diabetes-causing effects of mutations at residue K170 (K170N and K170R; Fig. 3) within this region (29).

Since all proteins are evolutionarily tailored to generate specific functions, it follows that disease-causing mutations are most likely to cause loss of the specific function. In the present context, loss of high-affinity inhibition by ATP, either directly or indirectly, is thus the most likely mechanism to underlie the net "gain of function" that underlies neonatal diabetes. However, it is conceivable that spontaneous mutations may also cause an increase in an activating function, such as an increase in the MgADP affinity of SUR1. Alternately, mutations could increase the affinity for PIP_2 or other phospholipids, which serve as

activators of the channel. In this regard, one or two rare mutations engineered into SUR1 (83,92) increase activation by MgADP, and they could potentially appear spontaneously. Mutations of Kir2.1, a related K channel subunit, underlie Andersen's syndrome, which is characterized by dysmorphic facial features, epilepsy, and cardiac arrhythmias (93,94). It is argued that these mutations alter Kir2.1 channel function by modulating the phospholipids sensitivity (94). Similarly, many mutations in K_{ATP} are likely to affect apparent PIP_2 sensitivity by changes in intrinsic open-state stability (54) or by change in affinity. Although none have been reported, it remains possible that neonatal diabetes could also result from gain of function due to an increased affinity for PIP_2.

Perspectives and prospects. The 10-year effort that first yielded the molecular basis of K_{ATP} channel activity (77,79) permitted the generation of animal models of channel dysfunction (11) and paved the way to genetic determination of predisposing polymorphisms in type 2 diabetes (41,45,46,95) and disease-causing mutations in both CHI (5) and neonatal diabetes (27,29,33). This in turn has led to an immediate understanding of the likely molecular basis of permanent neonatal diabetes and to improved therapy (31,36). This progression is a dramatic demonstration of the power of multidisciplinary biology in disease analysis. The current results elucidate the molecular basis of a traumatic neonatal disease and exciting possibilities for improvement in treatment options.

K_{ATP} channel may be involved in a whole spectrum of diabetes. For the E23K polymorphism, the effect on nucleotide sensitivity is modest, and only in the proper genetic and environmental background may channel overactivity appreciably influence β-cell function. Mutations resulting in greater gain of function may still be found to underlie childhood diabetes, while mutations that result in more significant gain of function are likely to give rise to an earlier and more severe form of diabetes, as in permanent neonatal diabetes. In the most severe cases, K_{ATP} channel overactivity in extrapancreatic tissue likely contributes to multiorgan syndromic permanent neonatal diabetes. Sulfonylureas may provide dramatic improvement in therapeutic options for neonatal diabetes (27,31,33), but the sulfonylurea-desensitizing effect of open-state–stabilizing mutations (39,56,96) and the possibility of secondary nonelectrical consequences heed caution regarding whether this is a panacea.

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