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ATP-sensitive K⁺ channels and disease: from molecule to malady

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Ashcroft FM. ATP-sensitive K⁺ channels and disease: from molecule to malady. Am J Physiol Endocrinol Metab 293: E880–E889, 2007. First published July 24, 2007; doi:10.1152/ajpendo.00348.2007.—This essay is based on a lecture given to the American Physiological Society in honor of Walter B. Cannon, an advocate of homeostasis. It focuses on the role of the ATP-sensitive potassium K⁺ (K<sub>ATP</sub>) channel in glucose homeostasis and, in particular, on its role in insulin secretion from pancreatic β-cells. The β-cell K<sub>ATP</sub> channel comprises pore-forming Kir6.2 and regulatory SUR1 subunits, and mutations in either type of subunit can result in too little or too much insulin release. Here, I review the latest information on the relationship between K<sub>ATP</sub> channel structure and function, and consider how mutations in the K<sub>ATP</sub> channel genes lead to neonatal diabetes or congenital hyperinsulinism.

Kir6.2; SUR1; neonatal diabetes; hyperinsulinism

WALTER BRADFORD CANNON (1871–1945) was an eminent American physiologist who pioneered the concept of homeostasis. In his seminal text, The Wisdom of the Body, he described it as “the coordinated physiological reactions which maintain most of the steady states in the body.” Among these steady states, one of the most important is that of the blood glucose concentration. In nondiabetic individuals, the fasting blood glucose level is at 4–5 mM. If glucose drops below ~1–2 mM, the brain is starved of fuel, causing cognitive impairment and ultimately loss of consciousness. Long-term elevation of the blood glucose concentration (>7 mM) is also dangerous, as it results in glycation of membrane proteins and gives rise to a host of complications that include retinopathy, nephropathy, peripheral neuropathy, and cardiac disease. Glucose homeostatic mechanisms ensure that the blood glucose fluctuations which occur inevitably after every carbohydrate meal are only transient. When these mechanisms fail, the result is diabetes mellitus or its converse, hyperinsulinism. This essay is based on the American Physiological Society’s 2007 lecture in honor of Walter B. Cannon and takes as its theme glucose homeostasis in health and disease.

Insulin plays a crucial role in blood glucose homeostasis because it is the only hormone capable of reducing the blood glucose level; in contrast, many hormones can increase blood glucose levels. Diabetes mellitus results if insufficient insulin is produced to meet the needs of the body. The term refers to a range of conditions all of which are characterized by an elevated blood glucose level but which may have different etiologies. Several different forms of the disease are recognized.

Type 1 diabetes typically presents in childhood as an autoimmune attack on the pancreatic β-cells that results in their complete destruction; consequently, the patient must take insulin for the rest of their life. It accounts for more than 10% of all diabetes and will not be considered further here. Type 2 diabetes, which is reaching epidemic proportions in Western societies and is predicted to affect 300 million people worldwide by 2025, is a very different disease (42). It is normally associated with both inadequate insulin secretion and impaired insulin action. Type 2 diabetes is a polygenic disease that results from a combination of gene variants in many different genes, each of which individually has only a small effect. Some of these genes have recently been identified by genome-wide scans (20, 80, 90). There are also several different forms of monogenic diabetes that result from single gene mutations (22, 24, 32, 76). These disorders are rare, but their study has provided important insights into insulin secretion in both health and disease and led to identification of some of the genes that contribute to type 2 diabetes.

This essay focuses on the ATP-sensitive potassium (K<sub>ATP</sub>) channel, which plays a key role in blood glucose homeostasis. Mutations in the genes that encode this channel can lead to neonatal diabetes or congenital hyperinsulinism. There are many excellent and comprehensive recent reviews on the K<sub>ATP</sub> channel and its role in human disease (3, 13, 32, 39, 54, 58). In this essay, therefore, I have taken a different approach and instead provide a personal perspective of how we reached our current state of understanding of K<sub>ATP</sub> channel function. The story of the K<sub>ATP</sub> channel is a long one. More than 20 years elapsed between the discovery that it regulates insulin secretion and the introduction of a new therapy for children with neonatal diabetes. Translational research, no matter how fast the final stages may appear, usually takes a long time.

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**K<sub>ATP</sub> Channels: the Early Days**

In 1983, when I first entered the field, it was well established that glucose metabolism was required to stimulate insulin release (7). It was also known that Ca<sup>2+</sup>-dependent β-cell electrical activity was essential, as this led to an influx of calcium that was the trigger for insulin secretion (63). Technically demanding studies in which sharp microelectrodes were used to record from β-cells within isolated islets had shown that in the absence of glucose the β-cell was electrically silent, with a resting potential of around −70 mV and that glucose produced a gradual membrane depolarization that triggered electrical activity (reviewed in Refs. 6 and 35). What was not so clear was how glucose initiated this depolarization. However, there was a clue: work by Janove Sehlin and Inge-Bert Taljedal (74) and subsequently by Jean-Claude Henquin (33) had revealed that glucose metabolism reduced efflux of 86Rb (a congener for K<sup>+</sup>), suggesting that closure of a K<sup>+</sup> channel was involved.

The advent of the high-resolution patch clamp technique in 1981 (31) enabled this K<sup>+</sup> channel to be identified, and I used this method on single β-cells dispersed from pancreatic islets (4). As we were looking for a channel regulated by glucose metabolism, we used cell-attached membrane patches to ensure that β-cell metabolism remained intact. We also added glucose to the bath solution, so that any effect of glucose on the activity of ion channels in the patch of membrane under the recording pipette had to be mediated via an intracellular route. Finally, we filled the pipette with a high K<sup>+</sup> solution to shift the K<sup>+</sup> equilibrium potential and enable single K<sup>+</sup> channel currents to be recorded at the resting potential of the β-cell. Our studies led to discovery of a K<sup>+</sup> channel that was active at the resting potential and closed by glucose metabolism (4).

Intriguingly, the single-channel conductance and kinetics of this K<sup>+</sup> channel were very similar to those of a K<sup>+</sup> channel identified in cardiac myocytes the previous year that was blocked by elevation of intracellular ATP (61). A similar K<sub>ATP</sub> channel had also just been described by Dan Cook and Nick Hales in inside-out patches excised from pancreatic β-cells (10). Within a year of its discovery, Patrik Rorsman and Gerd Trube had shown the glucose-sensitive K<sup>+</sup> channel was identical to the K<sub>ATP</sub> channel (70).

Thus, by 1985, there was a consensus model for glucose-dependent insulin secretion (Fig. 1). This postulated that insulin release is initiated by elevation of the intracellular Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]), which is mediated by Ca<sup>2+</sup> influx through voltage-gated Ca<sup>2+</sup> channels in the plasma membrane. In the unstimulated β-cell, K<sub>ATP</sub> channels are open, and K<sup>+</sup> influx through these channels keeps the membrane potential at a negative level where voltage-gated Ca<sup>2+</sup> channels are closed. When the plasma glucose concentration rises, glucose uptake and metabolism by the pancreatic β-cell are enhanced, leading to closure of the K<sub>ATP</sub> channels. This causes a membrane depolarization that triggers opening of voltage-gated Ca<sup>2+</sup> channels and Ca<sup>2+</sup>-dependent electrical activity. The consequent Ca<sup>2+</sup> influx stimulates insulin release.

Sulfonylurea drugs were soon shown to stimulate insulin secretion by closing K<sub>ATP</sub> channels directly, thereby bypassing the metabolic steps in stimulus-secretion coupling (27, 87). Despite the fact that their target was unknown, these drugs had been used to treat type 2 diabetes since the 1950s—and they are still used today. The story of their serendipitous discovery has been told elsewhere (34).

For many years, the product of glucose metabolism that regulates K<sub>ATP</sub> channel activity was hotly contested. While it was very clear that ATP shut the channel in inside-out patches, the ATP sensitivity was so high that in the intact β-cell the K<sub>ATP</sub> channel should always be closed (5, 10). This did not agree with the observation that the channel was open in β-cells exposed to glucose-free solution (4). This suggested the presence of additional channel regulators. In fact, the regulation of K<sub>ATP</sub> channel turned out to be extremely complex. It is the target for numerous cytosolic compounds, including nucleotides (5, 10, 14, 40), lipids such as phosphatidylinositol bisphosphate (9, 17, 79), and long-chain acyl-CoAs (26, 48). Of particular importance is MgADP, which stimulates channel activity and can reactivate channels closed by ATP (14, 40).

In the early days, many biochemists had a certain resistance to the idea that ATP could act as a signaling molecule—reasonably enough, because in most cells ATP levels are very stable. We now know, however, that ATP levels in β-cells do change in response to glucose metabolism: from about 2 mM to 4 mM when glucose is increased from 0 to 10 mM (11, 84). Thus, it is now generally believed that changes in adenine nucleotide concentrations are largely responsible for mediating the effects of glucose metabolism on K<sub>ATP</sub> channel activity. At low glucose concentrations, where metabolism is low, the stimulatory effects of MgADP predominate, whereas when glucose metabolism is stimulated the inhibitory action of ATP is favored.

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**Fig. 1. Role of ATP-sensitive K<sup>+</sup> (K<sub>ATP</sub>) channels in insulin secretion.**

*A:* when metabolism is low, K<sub>ATP</sub> channels are open, keeping the membrane hyperpolarized and voltage-gated Ca<sup>2+</sup> channels closed, so that [Ca<sup>2+</sup>]<sup>2</sup> remains low and insulin secretion is prevented. **B:** when metabolism increases, ATP increases and MgADP falls, so that K<sub>ATP</sub> channels shut. This triggers depolarization of the β-cell membrane potential, opening of voltage-gated Ca<sup>2+</sup> channels, Ca<sup>2+</sup> influx, and exocytosis of insulin granules.
The K$_{\text{ATP}}$ channel dominates the $\beta$-cell resting potential, and there is a highly nonlinear relationship between the K$_{\text{ATP}}$ channel conductance and the $\beta$-cell membrane potential (85). This means that, close to the threshold for electrical activity, very small changes in K$_{\text{ATP}}$ conductance have marked effects on electrical activity and insulin secretion. It was therefore evident right from the start that tiny changes in K$_{\text{ATP}}$ conductance resulting from mutations in K$_{\text{ATP}}$ channel genes, or from impaired metabolic regulation of K$_{\text{ATP}}$ channel activity, would result in altered insulin secretion. Demonstration that this was the case, however, had to await the cloning of genes encoding the K$_{\text{ATP}}$ channel.

This took time; indeed, it was not until 10 years after its discovery that the $\beta$-cell K$_{\text{ATP}}$ channel was cloned. One reason it was unusually difficult was that it turned out that the K$_{\text{ATP}}$ channel is, in fact, a complex of two different proteins (37, 71). The pore-forming subunit, known as Kir6.2, is a member of the inwardly rectifying K$^+$ channel family (37, 71). This associates in a 4:4 complex (38) with a much larger regulatory subunit, the sulfonylurea receptor SUR1, which belongs to the ABC family of ATP-binding cassette transporters (1). Cloning of Kir6.2 was hard enough, but cloning of SUR1 by Joe Bryan and colleagues was a tour de force, requiring purification of the protein itself followed by NH$_2$-terminal sequencing and screening of cDNA libraries (1). These were the heroic days when one could not simply order the clone!

**Current View of K$_{\text{ATP}}$ Channel Structure and Function**

Cloning of the genes encoding Kir6.2 (KCNJ11) and SUR1 (ABCC8) enabled the relationship between the structure and function of the K$_{\text{ATP}}$ channel to be addressed by electrophysiological or biochemical analysis of heterologously expressed wild-type and mutant K$_{\text{ATP}}$ channels.

Such studies revealed that the K$_{\text{ATP}}$ channel is an octameric complex of four Kir6.2 subunits and four SUR1 subunits (38). Neither subunit can reach the plasma membrane in the absence of its partner, because each possesses an endoplasmic reticulum (ER) retention motif that must be masked by the other subunit (91). This ensures that only fully functional K$_{\text{ATP}}$ channels are trafficked to the surface membrane. We serendipitously discovered, however, that deletion of the last 35 or so residues of Kir6.2 enables it to be expressed at the membrane surface in the absence of SUR1 (88) [it deletes the ER retention signal (91)]. This allowed the intrinsic properties of Kir6.2 to be distinguished from those conferred by SUR1 (88).

Specific functions could then be assigned to the individual subunits. Metabolic regulation of K$_{\text{ATP}}$ channel activity was found to be mediated by both Kir6.2 and SUR1 subunits. The site to which ATP binds to close the channel lies on Kir6.2 (88), whereas MgADP binding to SUR1 stimulates channel activity (28, 59, 88). Although MgATP can also stimulate K$_{\text{ATP}}$ channel activity via interaction with SUR1 (29), it appears that it must first be hydrolyzed to MgADP (92). SUR thus functions as a second metabolic sensor, endowing the K$_{\text{ATP}}$ channel with an exquisite sensitivity to changes in adenine nucleotide concentrations. It also serves as a target for the inhibitory sulfonylurea drugs and the stimulatory K channel openers, both of which act by binding to SUR and thereby influence opening and closing (gating) of the Kir6.2 pore (1, 88).

A key question is: where are the binding sites for drugs and nucleotides on the K$_{\text{ATP}}$ channel and how does ligand binding lead to changes in channel gating? A mechanistic understanding of this question would be greatly facilitated by knowledge of the atomic structure of the channel. Unfortunately, this is not yet available. Currently, there is only a low-resolution structure of the purified complex (53). This reveals that the K$_{\text{ATP}}$ channel assembles as a central tetrameric Kir6.2 pore surrounded by four SUR1 subunits, but sheds no light on the binding sites.

Some information on the location of the binding sites can, however, be gleaned from homology modeling and mutagenesis studies. Like other ABC proteins, SUR1 has two large cytosolic domains that have consensus sequences for ATP binding and hydrolysis. Mutations in these nucleotide-binding domains (NBDs) impair radiolabeled ATP binding (51) and channel activation by Mg-nucleotides (28). The high conservation of amino acid sequence and overall folds of the NBDs across ABC proteins suggests that homology models of the NBDs of SUR1 based on ABC crystal structures may be a reasonable approximation to reality. However, the transmembrane domains of SUR1 are too divergent to model accurately.

Shozeb Haider, Mark Sansom, and I have produced a homology model of Kir6.2 (2) based on the crystal structures of the transmembrane domains of a bacterial Kir channel (47) and the cytosolic domain of an eukaryotic Kir channel (60). We then used automated docking to probe for the ATP-binding site (2). The putative binding pocket lies at the interface between the cytosolic domains of adjacent Kir6.2 subunits, with residues in the COOH terminus of one subunit forming the main binding pocket and residues in the NH$_2$ terminus of the adjacent subunit also contributing. This location is in agreement with a substantial body of mutagenesis data. It later turned out that many Kir6.2 mutations which cause neonatal diabetes lie within the putative ATP-binding site (3, 23, 50, 77, 82), which adds further strength to our argument that this region is involved in ATP binding.

**Identification of Disease-Causing K$_{\text{ATP}}$ Channel Mutations**

The discovery of mutations in the K$_{\text{ATP}}$ channel subunits SUR1 and Kir6.2 that cause congenital hyperinsulinism of infancy (CHI) followed rapidly upon cloning of the genes (57, 86). This disorder affects about 1 in 50,000 live births and is characterized by continuous and unregulated insulin secretion in the face of very low blood glucose levels (13). Patients with this disorder usually present at birth or shortly afterwards. In some cases, their blood glucose levels fall so low that they suffer brain damage as a consequence.

More than 100 CHI mutations in SUR1 have now been described, distributed throughout the whole protein, and several mutations in Kir6.2 (13, 24). Many of these mutations result in the total loss of K$_{\text{ATP}}$ channels in the plasma membrane due to abnormalities in gene expression, protein synthesis, maturation, assembly, or membrane trafficking. Other SUR1 mutations impair the ability of the channel to respond to metabolic activators such as MgADP; consequently the channels are always closed. All CHI mutations are therefore loss-of-function mutations that result in permanent depolarization of the $\beta$-cell membrane and thereby continuous Ca$^{2+}$ influx and insulin secretion.
Of Mice and Men: Mouse Models of Hyperinsulinism and Diabetes

Mouse models have traditionally been used to probe the molecular mechanism of human disorders. The K<sub>ATP</sub> channel is no exception, and both gain- and loss-of-function models have been generated, mainly by Susumu Seino, Colin Nichols, and their colleagues (54). These have yielded important insights into human diabetes and CHI.

That gain-of-function K<sub>ATP</sub> channel mutations cause severe neonatal diabetes was first described in mice (44), four years before it was shown in humans (23). Because the mutant Kir6.2 transgene was targeted to the β-cell, extrapancreatic symptoms (such as those found in some patients with Kir6.2 mutations) were absent. No change in insulin content or β-cell number was observed, but serum insulin levels were extremely low, as expected from the reduced ATP sensitivity of the K<sub>ATP</sub> channel (44). Interestingly, mice in which the mutant transgene was targeted to the heart had no obvious cardiac symptoms (43), as was subsequently found for patients with gain-of-function K<sub>ATP</sub> channel mutations (3, 23). Mice in which the mutant transgene was expressed at much lower levels usually exhibited impaired glucose tolerance rather than diabetes (46). Thus, as in humans (3, 32), mice exhibit a spectrum of diabetes phenotypes that correlates with extent of K<sub>ATP</sub> channel activity.

As predicted, the pancreatic β-cells of mice lacking functional K<sub>ATP</sub> channels were depolarized and had elevated basal [Ca<sup>++</sup>]; (45, 55, 56, 73). Glucose-stimulated insulin secretion from isolated islets was absent, confirming that K<sub>ATP</sub> channels are crucial for insulin release. But the mouse models also produced some surprises. Animals in which the K<sub>ATP</sub> channel was functionally inactivated by a dominant negative Kir6.2 mutation (Kir6.2G132S) exhibited hypoglycemia and hyperinsulinemia as neonates but subsequently developed hyperglycemia due to substantial β-cell loss (56). Similarly, when either Kir6.2 or SUR1 was genetically deleted, adult mice had reduced insulin secretion and were normoglycemic (55, 73). Remarkably, however, mice expressing a different dominant negative Kir6.2 mutation exhibited hyperinsulinism as adults (45) and showed no β-cell loss. It was hypothesized that the very different phenotype of these mice might be because K<sub>ATP</sub> channel activity was only partially suppressed (30% of β-cells were unaffected). Most patients with severe CHI undergo sub-total pancreatectomy as infants to control their hyperinsulinism, but nonsurgically treated patients sometimes progress to glucose intolerance or diabetes (36). The mouse models suggest that this may reflect a gradual β-cell loss, which happens more slowly if suppression of K<sub>ATP</sub> channel activity is less severe.

It is also clear from Fig. 1 that gain-of-function mutations in K<sub>ATP</sub> channel genes would be expected to decrease insulin release and lead to diabetes. Such mutations were looked for in patients with type 2 diabetes as soon as the gene was cloned, and as early as 1996 we identified a commonly occurring variant at residue 23 of Kir6.2 (E23K) (72). Excitingly, the K variant occurred with a greater frequency in the diabetic population, but in our studies the difference was not significant (the sample of 100 patients was far too small), so no conclusion could be drawn. It is now known that this polymorphism does indeed convey an increased risk of type 2 diabetes. However, the increase in risk is modest—the odds ratio is 1.2—and it required large population studies involving thousands of patients and controls to demonstrate the association (25, 90).

It was obvious, even when we were genotyping type 2 diabetic patients for mutations in Kir6.2, that we were really looking at the wrong population. Type 2 diabetes typically develops late in life, whereas a severe monogenic disorder would be expected to manifest at birth, like CHI. However, at that time, all the clinicians I consulted told me that neonatal diabetes did not exist. Subsequently it turned out this was wrong: such patients were extremely rare, and most clinicians had simply not come across them.

Andrew Hattersley, however, knew that such patients existed despite their rarity. He also recognized that studies of patients with monogenic diabetes could potentially identify new genes that regulated insulin secretion and provide novel insights into type 2 diabetes. He therefore set up an international collection of patients with neonatal diabetes and began screening their DNA for mutations in candidate genes. I shall never forget the telephone call I received from Andrew and Anna Gloyn telling me they had found the first mutation in Kir6.2 associated with neonatal diabetes. It was a truly exciting day. Equally exciting was our subsequent discovery that the mutation impaired ATP inhibition of the K<sub>ATP</sub> channel (23).

Current Understanding of the Role of K<sub>ATP</sub> Channels in Neonatal Diabetes

Neonatal diabetes (ND) is now defined as diabetes presenting within the first six months of life (15). It affects about 1 in 200,000 live births and is characterized by severe hyperglycemia, which may be either permanent or transient. Why some patients show neonatal diabetes that subsequently remits and may later relapse again (19, 21, 32) is not understood. Neonatal diabetes is sometimes accompanied by mental and motor developmental delay, epilepsy, and muscle weakness, a condition known as DEND syndrome (32). Patients with intermediate DEND (iDEND) syndrome have neonatal diabetes coupled with delayed speech and walking and may also show muscle weakness (32).

Neonatal diabetes is caused by mutations in a number of different genes, but studies over the last three years have shown that the commonest mutations are in KCNJ11 (Kir6.2) and ABCC8 (SUR1) (3, 8, 16, 19, 23, 32, 65). Patients with ND-Kir6.2 mutations are all heterozygotes (3, 32) whereas ND-SUR1 mutations are genetically more heterogeneous, with heterozygotes, homozygotes, and compound heterozygotes all being described (8, 16, 65). In the case of Kir6.2 mutations, there is a reasonable correlation between genotype and the clinical phenotype (3, 32). It is still too early to know whether this is also the case for SUR1 mutations.

To date, over 30 gain-of-function mutations in Kir6.2 associated with neonatal diabetes have been identified, the most prevalent being at residues R201 and V59 (19, 32). These
mutations cluster around the putative ATP-binding site or lie in regions of the protein involved in channel gating, such as the slide helix, the cytosolic mouth of the channel, or the gating loops that link the ATP-binding site to the slide helix (Fig. 2). They may also affect residues involved in intrasubunit interactions. Functional analysis of ND-Kir6.2 mutations has provided useful insights into the working of the KATP channel. More recently, gain-of-function mutations have been also identified in the SUR1 subunit (8, 16, 65). These are dispersed throughout the protein sequence but are particularly concentrated in the first five transmembrane helices and connecting loops, the third cytosolic linker, and in NBD2.

All Kir6.2 and SUR1 ND mutations that have been functionally analyzed to date are gain-of-function mutations that produce an increase in the resting whole cell KATP current. This is predicted to reduce glucose-dependent depolarization of the β-cell membrane, thus preventing activation of Ca2+ currents, electrical activity, Ca2+ influx, and insulin secretion (76).

**Functional Effects of Kir6.2 Mutations Causing Neonatal Diabetes**

The effects of more than 20 ND-Kir6.2 mutations on KATP channel properties have been explored by heterologous expression of recombinant channels (3, 21, 32, 50, 64, 67, 68, 77, 82). Because all patients are heterozygotes, the heterozygous state has been simulated by coexpression of wild-type and mutant Kir6.2 with SUR1. The increase in KATP current caused by ND-Kir6.2 mutations results from an impaired ability of MgATP to close the KATP channel. ATP concentration-response curves reveal that Kir6.2 mutations may shift the ATP concentration at which channel inhibition is half-maximal to higher ATP concentrations and/or may lead to some KATP current remaining unblocked even at saturating ATP concentrations. In all cases, however, the magnitude of the KATP current at physiologically relevant ATP concentrations (i.e., that expected in the presence of glucose) is increased. All ND-Kir6.2 mutations increase the KATP current at 3 mM MgATP at least 20-fold, and those mutations that cause DEND syndrome have the greatest effect (Fig. 3). There appears to be no clear correlation, however, between the magnitude of the KATP current and whether the mutation causes permanent or relapsing-remitting neonatal diabetes.

Neonatal diabetes mutations that lie within the putative ATP-binding site of Kir6.2 are considered to impair channel inhibition at Kir6.2 by interfering with nucleotide binding (3, 32, 50, 64, 77). While the electrophysiological data are entirely consistent with this view, this has yet to be demonstrated in biochemical studies. Many mutations, however, appear to affect ATP inhibition indirectly by altering KATP channel gating (21, 64, 67, 68). These mutations stabilize the intrinsic open state of the channel (i.e., that in the absence of ATP), which shifts the gating equilibrium in the presence of ATP towards channel opening and thus indirectly reduces the channel ATP
hyperpolarized even when blood glucose levels are elevated, thereby keeping voltage-gated Ca\(^{2+}\) channels closed and preventing the channel from closing in response to metabolically generated changes in adenine nucleotides. Consequently, the KATP current at physiologically relevant ATP concentrations is ineffective as intracellular Ca\(^{2+}\) levels are low.

**Extrapancreatic Effects of ND Mutations**

Kir6.2 is widely expressed throughout the body, being found in heart, skeletal muscle, various endocrine cells, peripheral axons, and multiple types of brain neurons (37, 41, 71). Gain-of-function mutations in Kir6.2 are therefore expected to reduce the electrical excitability of these cells. This can account for the neurological and motor defects found in DEND patients: the epilepsy can be explained as a result of reduced activity of inhibitory neurons.

That much larger K\(_{\text{ATP}}\) currents appear to be required to affect extrapancreatic tissues may reflect differences both in cell metabolism and in the complement of ion channels that regulate the cell membrane potential. The β-cell is unusual in that K\(_{\text{ATP}}\) channel activity dominates the resting membrane potential and in that channel activity is sensitive to changes in extracellular glucose (in many cell types, KATP channels open only during ischemia).

Although Kir6.2 is expressed in the heart (37, 71) and forms the pore of the cardiac K\(_{\text{ATP}}\) channel (18), no obvious cardiac abnormalities have been reported in patients carrying Kir6.2 mutations. Similarly, mice engineered to carry gain-of-function Kir6.2 mutations that are expressed only in the heart are not substantially affected (43). This conundrum may be explained by the fact that coexpression of mutant Kir6.2 with the cardiac type of SUR (SUR2) produces a very much smaller increase in the K\(_{\text{ATP}}\) current at physiologically relevant ATP concentrations than coexpression with SUR1 (83). This seems to be because Mg-nucleotide activation is enhanced by SUR1 but not SUR2A (49, 52, 83).

**Implications for Therapy of ND Patients**

Before the discovery that ND can be caused by mutations in the K\(_{\text{ATP}}\) channel, it was assumed these patients suffered from an unusually early onset form of type 1 diabetes. Thus they were treated with insulin. The discovery that ND patients possess gain-of-function mutations in K\(_{\text{ATP}}\) channel genes immediately suggested another therapy: sulfonylureas should stimulate insulin secretion in these patients by closing their K\(_{\text{ATP}}\) channels (Fig. 4). Andrew Hattersley’s group were the first to introduce sulfonylurea therapy for patients with Kir6.2 mutations that cause neonatal diabetes (23) and have now shown that over 90% of these “insulin-dependent” patients can be managed by sulfonylureas alone (62). Importantly, not only is their quality of life enhanced but there is also a significant improvement in their blood glucose control. Fluctuations in blood glucose are reduced (93), and there is a marked decrease in their HbA1C levels (which provides a measure of their average blood glucose level during the proceeding weeks) (62). The improvement in blood glucose would be expected to reduce the risk of diabetic complications (12, 89).

As in nondiabetics, oral glucose is much more effective than intravenous glucose at eliciting insulin secretion in patients treated with sulfonylureas (62). This is because oral glucose triggers the release of incretins such as glucagon-like peptide-1 (GLP-1) and gastrointestinal peptide (GIP) from the gut. These hormones are unable to elicit insulin secretion alone, because they do not close K\(_{\text{ATP}}\) channels. However, if intracellular Ca\(^{2+}\) has been elevated by closure of K\(_{\text{ATP}}\) channels, they are able to amplify insulin secretion (6). As expected, incretins have no effect in ND patients with K\(_{\text{ATP}}\) channel mutations prior to sulfonylurea therapy, because blood glucose levels do not shut K\(_{\text{ATP}}\) channels (62) (Fig. 4A). Following sulfonylurea treatment and K\(_{\text{ATP}}\) channels closure, however, they are able to amplify insulin secretion (Fig. 4B).

Sulfonylureas are very effective at treating patients with Kir6.2 mutations that cause neonatal diabetes without neurological complications. Functional analysis has shown that the mutations these patients carry have little effect on sulfonylurea block on K\(_{\text{ATP}}\) channels (62, 93). In contrast, sulfonylureas were not effective in patients with Kir6.2 mutations that cause

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**Fig. 4.** Sulfonylureas stimulate insulin secretion in neonatal diabetes due to K\(_{\text{ATP}}\) channel mutations. A: gain-of-function mutations in K\(_{\text{ATP}}\) channel subunits prevent the channel from closing in response to metabolically generated changes in adenine nucleotides. Consequently, the β-cell membrane remains hyperpolarized even when blood glucose levels are elevated, thereby keeping voltage-gated Ca\(^{2+}\) channels closed and preventing insulin secretion. Incretins are ineffective as intracellular Ca\(^{2+}\) levels are low. B: sulfonylureas bypass the metabolic steps and bind directly to the K\(_{\text{ATP}}\) channel, causing it to close. This triggers depolarization of the β-cell membrane potential, opening of voltage-gated Ca\(^{2+}\) channels, Ca\(^{2+}\) influx, and insulin secretion in patients with gain-of-function mutations in K\(_{\text{ATP}}\) channels. Incretins are also now effective.
DEND syndrome, presumably because these particular mutations strongly impaired the ability of sulfonylureas to block the $K_{\text{ATP}}$ channel (62).

Although it is still early days, there are an increasing number of cases in which sulfonylureas have been able to ameliorate the muscle weakness found in patients with iDEND syndrome and, to a variable extent, their motor and mental developmental delay (78, 81). Thus, while insulin cannot ameliorate the extrapancreatic effects of ND mutations, sulfonylureas may be able to do so because they can close overactive $K_{\text{ATP}}$ channels in all tissues to which they have access.

$K_{\text{ATP}}$ Channels and Glucose Homeostasis: a Question of Balance

In summary, $K_{\text{ATP}}$ channel activity makes an important contribution to glucose homeostasis. When these channels are shut, insulin is secreted, and when they are open, insulin release is prevented. $K_{\text{ATP}}$ channel activity is tightly regulated by the $\beta$-cell metabolism, and if this coupling is disturbed, insulin secretion is perturbed. Thus, diabetes results when $K_{\text{ATP}}$ channels fail to close in response to enhanced metabolism, and congenital hyperinsulinism results when $K_{\text{ATP}}$ channels remain open even when plasma glucose levels fail. Very small changes in $K_{\text{ATP}}$ channel activity are sufficient to produce marked changes in insulin secretion. As Walter Cannon reminds us, homeostasis is a finely balanced affair.

Beyond the Frontiers

Twenty-three years on from the discovery that the $K_{\text{ATP}}$ channel is important in insulin secretion, many key questions remain unanswered and there is much to do. The atomic structure of the individual subunits, Kir6.2 and SUR1, have still to be resolved, something that is essential if we are to unequivocally identify the binding sites for drugs and nucleotides. The structure of the whole octameric $K_{\text{ATP}}$ channel complex would be even more valuable as this might help clarify how interaction of Mg-nucleotides or drugs with SUR1 is transmitted to Kir6.2 to modulate K$^+$ flux through the channel pore.

Precisely how most SUR1 mutations that cause neonatal diabetes influence $K_{\text{ATP}}$ channel activity is yet to be discovered. Likewise, how the E23K polymorphism in Kir6.2 enhances the risk of type 2 diabetes is still unclear. Functional studies have yielded conflicting results, with both a decrease (75) and an increase (69) in ATP inhibition being reported. Furthermore, it is puzzling that the reported reduction in ATP sensitivity (75) is similar to that found for mutations that cause permanent neonatal diabetes (67, 77, 82).

At the clinical level, it is important to determine the extent to which sulfonylureas can ameliorate the extrapancreatic symptoms of patients with iDEND and DEND syndrome. There is also a question of the choice of sulfonylurea: it seems logical that a SUR1-specific drug might be advisable for patients with SUR1 mutations, but would it also be beneficial in patients with Kir6.2 mutations? Why older PNDM patients respond less well to sulfonylurea therapy requires elucidation, as does the mechanism by which severe Kir6.2 mutations lead to motor and mental developmental delay, muscle weakness, and epilepsy. No doubt, mice carrying disease-causing mutations will be helpful in this respect.

These are but a few of the many questions in the field. Answering them may take another 20 years, but it is bound to be an exciting journey. For young scientists looking for a discipline that embraces both basic and clinical science, the $K_{\text{ATP}}$ channel has much to offer.

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REFERENCES

16. Ellard E, Flanagan SE, Girard CA, Patch AM, Harris LW, Parrish A, Edgfield EL, Mackay DJG, Proks P, Shimomura K, Haberland HS, Carson DJ, Shield JPF, Hattersley AT, Ashcroft FM. Permanent neonatal diabetes caused by dominant, recessive or compound heterozy-


