K<sub>ATP</sub> channels and insulin secretion: a key role in health and disease

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Abstract
This review summarizes advances in our understanding of the structure and function of the ATP-sensitive potassium (K<sub>ATP</sub>) channel of the pancreatic β-cell that have been made over the last 5 years. It discusses recent structural studies of the octameric K<sub>ATP</sub> channel complex and studies of the regulation of K<sub>ATP</sub> Channel activity by nucleotides. It then considers the molecular mechanism by which gain-of-function mutations in the Kir6.2 subunit of the K<sub>ATP</sub> channel reduce channel inhibition by ATP and thereby lead to neonatal diabetes, and how identification of these mutations has led to changes in therapy. Finally, it illustrates how mouse models of glucose intolerance or diabetes can provide fresh insight into β-cell function, using the C57BL/6J mouse, whose glucose intolerance arises from mutations in nicotinamide nucleotide transhydrogenase, as an example.

Introduction
K<sub>ATP</sub> channels (ATP-sensitive K channels) play a key role in insulin secretion by linking pancreatic β-cell metabolism to electrical activity of the plasma membrane [1]. Insulin release is triggered by a rise in [Ca<sup>2+</sup>]<sub>i</sub> (intracellular Ca<sup>2+</sup> concentration) that results from Ca<sup>2+</sup> influx through voltage-gated Ca<sup>2+</sup> channels in the β-cell plasma membrane. In the absence of metabolic substrates, such as glucose, the Ca<sup>2+</sup> channels are shut because the membrane is held at a hyperpolarized level by the activity of the K<sub>ATP</sub> channels. When blood glucose levels are elevated, glucose is rapidly equilibrated across the β-cell membrane via the GLUT2 glucose transporter. It is then phosphorylated by glucokinase, in a reaction that is rate-limiting for glucose metabolism. Subsequent metabolism by glycolysis and (most importantly) within the mitochondria leads to changes in the intracellular concentrations of adenine nucleotides that result in closure of the K<sub>ATP</sub> channels. This produces a membrane depolarization that opens voltage-gated Ca<sup>2+</sup> channels, initiating β-cell electrical activity and Ca<sup>2+</sup> influx. The subsequent rise in [Ca<sup>2+</sup>]<sub>i</sub> triggers insulin release.

 Sulphonylurea drugs, such as tolbutamide, stimulate insulin secretion by binding to and, closing, K<sub>ATP</sub> channels [2]. Thus, although they bypass β-cell metabolism, they subsequently stimulate the same chain of events as glucose. Mutations in K<sub>ATP</sub> channel genes, or defects in the metabolic regulation of the K<sub>ATP</sub> channel, lead to diabetes or hyperinsulinaemia [1], emphasizing further the critical role of the channel in insulin secretion.

Metabolic regulation of K<sub>ATP</sub> channels
Glucose metabolism regulates the activity of the K<sub>ATP</sub> channel via changes in the cytosolic concentrations of the adenine nucleotides ATP and MgADP, which inhibit and stimulate channel activity respectively. A long-standing puzzle has been that, in isolated membrane patches, the K<sub>ATP</sub> channel is fully blocked by 1 mM ATP [3], yet significant channel activity can be recorded from cell-attached patches on β-cells where [ATP]<sub>i</sub> (intracellular ATP concentration) is predicted to be 1–5 mM [4]. Recent studies, however, have resolved this conundrum by showing that the ATP sensitivity is much lower in permeabilized cells and compatible with measured [ATP]<sub>i</sub> [5]. This results from the stimulatory effect of Mg-nucleotides, which shifts the concentration–inhibition curve to higher ATP levels. For unknown reasons, this shift is less in the excised patch than intact cell. Current evidence suggests that changes in [ATP]<sub>i</sub> and [MgADP]<sub>i</sub> (intracellular MgADP concentration) are sufficient to explain the level of channel activity in β-cells exposed to glucose-free solutions, as well as the changes that occur when glucose is elevated.

K<sub>ATP</sub> channel structure
Structurally, K<sub>ATP</sub> channels are large hetero-octameric complexes of four pore-forming (Kir6.2) and four regulatory sulphonylurea receptor (SUR<sub>x</sub>) subunits [6]. A first glimpse of the three-dimensional structure of the complex has recently been provided by cryoelectron microscopy [7]. This reveals that the K<sub>ATP</sub> channel is a compact structure, roughly 18 nm in cross-section and 13 nm in height. Molecular models of Kir6.2 and SUR1 can be comfortably accommodated within the EM (electron microscopy) density map, with four Kir6.2 subunits forming a central pore that is surrounded by four SUR1 subunits (Figure 1A). Although the resolution of the EM structure is low (18 Å; 1 Å = 0.1 nm), insertion of the models makes novel predictions about the way in which the complex is organized. First, in the ligand-free state, the NBDs

Key words: ATP-sensitive potassium channel (K<sub>ATP</sub> channel), diabetes, insulin secretion, Kir6.2, nicotinamide nucleotide transhydrogenase (Nnt), sulphonylurea.

Abbreviations used: DEDG syndrome, syndrome in which development delay, epilepsy and muscle weakness accompany neonatal diabetes; EM, electron microscopy; K<sub>ATP</sub>, ATP-sensitive potassium channel; Kir6.2, pore-forming receptor subunit; NBD, nucleotide-binding domain; Nnt, nicotinamide nucleotide transhydrogenase; SUR<sub>x</sub>, sulphonylurea receptor subunits; [x]<sub>i</sub>, intracellular concentration of x.

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(nucleotide-binding domains) of adjacent SUR1 subunits come into close physical apposition, which suggests that the NBDs of adjacent subunits may interact functionally. Secondly, a cleft running between adjacent SUR1 subunits, just below the membrane, provides a route by which ATP may access its binding site on Kir6.2 (Figure 1B).

ATP closes the K<sub>ATP</sub> channel by binding to Kir6.2 [3]. A combination of molecular modelling and site-directed mutagenesis has putatively located the ATP-binding site on the outer surface of the protein, approx. 2 nm below the membrane, and at the interface between adjacent Kir6.2 subunits [8]. Precisely how ATP binding to one of the four ATP-binding sites on the Kir6.2 tetramer is translated into pore closure remains to be determined.

Both MgATP and MgADP increase K<sub>ATP</sub> channel opening by binding to NBDs of SUR1 [3,9–11]. However, it is widely believed that MgATP does not activate the channel directly; rather it must first be hydrolysed to MgADP [11]. Thus only the MgADP-bound state is thought to cause activation. Mutations that abolish ATP hydrolysis without affecting MgATP or MgADP binding would be invaluable for testing this idea, but, unfortunately, none have been identified to date. The NBDs are believed to co-operate in ATP binding/hydrolysis in a similar manner to those of other ABC (ATP-binding cassette) proteins, with residues from each NBD contributing to two distinct sites [12]. Current evidence suggests that most ATP hydrolysis occurs at Site 2 [11,13], which is formed largely from NBD2 with contributions from the linker region of NBD1 [12].

The rate of ATP turnover by the NBDs has recently been measured for the isolated NBDs as well as the whole complex. Both the rate of ATP hydrolysis and the affinity for ATP were significantly higher for the K<sub>ATP</sub> channel complex [7] than for the isolated NBDs [13]. It is possible that this reflects the fact that, in SUR1, the ATP-binding sites are heterodimers of NBD1 and NBD2, as explained above. In addition, it may be due to interactions between the NBDs of adjacent SUR1 subunits, such as those suggested by the structural studies.

In the intact cell, ATP concentrations can be as much as 10-fold higher than ADP concentrations, because ADP is buffered by intracellular proteins. Because ATP and ADP have similar affinities for the NBDs of SUR1 [14], the NBDs will always preferentially bind ATP. However, an increase in [MgADP], is expected to lower the off-rate of MgADP from NBD2 by product inhibition, thus enhancing channel activity when metabolism is low. Consistent with this idea, MgADP lowers the rate of ATP hydrolysis of the isolated NBD2 of SUR2 [11] and of the whole K<sub>ATP</sub> channel complex (F. M. Ashcroft, M. Mikhailov and H. de Wet, unpublished work).

Gain-of-function mutations in K<sub>ATP</sub> channel genes cause neonatal diabetes

Mutations in the gene encoding Kir6.2 (KCNJ11) are a common cause of neonatal diabetes [1]. This is because all mutations studied to date reduce the ability of ATP to inhibit the K<sub>ATP</sub> channel and simultaneously enhance the ability of MgATP to stimulate channel activity [15–17]. Some mutations are located in the putative ATP-binding site and probably decrease the channel’s ATP sensitivity by impairing ATP binding [11,12]. Others stabilize the open state of the channel: because ATP preferentially stabilizes the closed state of the channel, any increase in channel opening will indirectly reduce the ATP sensitivity [12]. While the mechanism by which Kir6.2 mutations reduce K<sub>ATP</sub> channel activity is straightforward to explain, precisely how these mutations affect the ability of Mg-nucleotide binding to SUR1 to increase channel activity remains a mystery.
Reduced ATP sensitivity correlates with increased $K_{ATP}$ current

Relationship between the fraction of $K_{ATP}$ current not blocked by 3 mM MgATP in the excised patch and the resting whole-cell current, expressed as a fraction of the maximal current recorded on metabolic inhibition with 3 mM azide. The line is a linear fit with a slope of 1.75; the regression coefficient is 0.92. The data are taken from [16,17,30]. TNDM, transient neonatal diabetes; TNDM, transient diabetic neonatal diabetes; PNDM, permanent neonatal diabetes; DEND, DEND syndrome; wild-type.

In general, mutations that affect Kir6.2 channel gating cause a greater decrease in ATP sensitivity and are associated with a more severe form of the disease known as DEND syndrome (in which development delay, epilepsy and muscle weakness accompany neonatal diabetes) [1,18]. There is a good correlation between the extent of block by ATP (in the presence of Mg$^{2+}$) and the increase in the whole-cell $K_{ATP}$ current [17] (Figure 2). In $\beta$-cells, an increased $K_{ATP}$ current will prevent glucose-induced depolarization, Ca$^{2+}$ entry and insulin secretion, thus producing diabetes. The neurological complications of DEND syndrome may arise because very large increases in $K_{ATP}$ current affect the activity of muscle and neuronal cells in which Kir6.2 is expressed.

Identification of Kir6.2 mutations as a cause of neonatal diabetes not only has provided new insights into the relationship between channel structure and function, but also has led to a new approach to therapy [18]. Before the discovery of Kir6.2 mutations, it was assumed that neonatal diabetes was a rare, early onset, form of Type I diabetes, and patients were routinely treated with insulin injection. Once it was recognized that the disease was caused by overactivity of $K_{ATP}$ channels, it was clear that sulphonylureas could be an alternative therapy. Many patients have now been successfully transferred to sulphonylurea drugs, and have even shown improved glucose control [18,19].

Impaired $\beta$-cell metabolism may underlie glucose intolerance and Type II diabetes

Type II diabetes has a polygenic and multifactorial aetiology, but the fact that sulphonylureas are effective in Type II diabetic subjects suggests that the insulin secretory defect lies before $K_{ATP}$ channel closure. Impairment of $K_{ATP}$ channel closure in response to glucose could arise either from an inability of the channel to recognize metabolic signals (e.g. ATP) or from a failure of $\beta$-cell metabolism. There is evidence from genetic studies that both can occur [20]. For example, a common gene variant (E23K) in Kir6.2 confers an enhanced susceptibility to Type II diabetes [21]. Some monogenic forms of diabetes result from mutations in genes that are thought to affect $\beta$-cell metabolism, such as those encoding glucokinase [22], mitochondrial DNA [23] and even transcription factors [24]. Common variants in these genes, and in other genes that modulate $\beta$-cell metabolism, have also been linked with Type II diabetes (see [20] for a review). It seems reasonable to speculate that the greater the number of such gene variants an individual possesses, the greater their risk of Type II diabetes.

Functional studies also suggest that an underlying metabolic defect contributes to impaired insulin secretion in human Type II diabetes. Compared with control islets, Type II diabetic islets have reduced glucose oxidation and insulin secretion [25]. Furthermore, although glucose closes $K_{ATP}$ channels and triggers electrical activity in pancreatic $\beta$-cells isolated from non-diabetic human cadaver organ donors [26], our recent studies show that glucose fails to do so as effectively in $\beta$-cells isolated from a patient with Type II diabetes. Tolbutamide, however, stimulated electrical activity, supporting the idea that, at least in this patient, metabolic regulation of $K_{ATP}$ channels was impaired. Because human Type II diabetes probably has multiple aetiologies, many more such studies are needed. Unfortunately, these are hampered by the scarcity of tissue.

In the absence of human $\beta$-cells, mouse models of diabetes and glucose intolerance can provide useful insights into the aetiology of human diabetes. One example is the C57BL/6J mouse strain, which shows impaired glucose tolerance resulting from reduced first- and second-phase insulin release [27]. Although the ATP sensitivity of the $K_{ATP}$ channel in C57BL/6J pancreatic $\beta$-cells is normal, glucose fails to close pancreatic $\beta$-cell $K_{ATP}$ channels or to elevate intracellular calcium and stimulate insulin secretion. This suggests a defect in glucose metabolism. Genetic mapping identified a loss-of-function mutation in the gene encoding Nnt (nicotinamide nucleotide transhydrogenase) as underlying these defects [27]. Nnt is a nuclear-encoded protein of the inner mitochondrial membrane which functions as a redox-driven proton pump, catalysing the reversible reduction of NADP$^+$ by NADH and conversion of NADH into NAD$^+$ [28].

Recent studies have shown that knockdown of Nnt by siRNA (small interfering RNA) in the insulin-secreting cell line MIN6 prevented the rise in [Ca$^{2+}$], produced by glucose and led to a dramatic reduction in insulin secretion [29]. However, tolbutamide was still effective, suggesting that, as in C57BL/6J $\beta$-cells, glucose fails to close $K_{ATP}$ channels when Nnt is non-functional. Similarly, studies on islets isolated from mice possessing ethynitrosourea-induced point mutations in Nnt revealed that both homozygous and heterozygous Nnt mutant mice were significantly glucose intolerant, and that their islets secreted less insulin [29]. Furthermore,
although basal ATP levels were normal in Nnt mutant mice β-cells, glucose failed to elevate ATP. This suggests that Nnt mutations impair mitochondrial ATP production in β-cells, and so prevent glucose-dependent closure of K<sub>ATP</sub> channels. Consequently, glucose-dependent β-cell electrical activity and insulin secretion are impaired.

Clearly, it will now be of interest to determine whether polymorphisms in the Nnt gene are associated with human Type II diabetes. However, even if this is not the case, analysis of models of diabetes can provide novel insights into suspected. This supports the idea that studies of mouse models of diabetes can provide novel insights into β-cell function.

**Conclusion**

During the last 5 years, there has been substantial progress in our understanding of how the K<sub>ATP</sub> channel contributes to the aetiology of diabetes, as this short review has illustrated. Nevertheless, much remains to be done. We still do not understand, for example, why the lysine variant at residue 23 of Kir6.2 predisposes individuals to Type II diabetes. Nor can we explain how almost all mutations in Kir6.2 that cause neonatal diabetes enhance the ability of Mg-nucleotide interactions with SUR1 to cause opening of the Kir6.2 pore. Likewise, the mechanism by which loss of Nnt function reduces ATP production, and thereby insulin secretion, remains to be established. With luck, illumination of all these points will come in the next 5 years.

**References**


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