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Review

Current understanding of K$_{ATP}$ channels in neonatal diseases: focus on insulin secretion disorders

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ATP-sensitive potassium (K$_{ATP}$) channels are cell metabolic sensors that couple cell metabolic status to electric activity, thus regulating many cellular functions. In pancreatic beta cells, K$_{ATP}$ channels modulate insulin secretion in response to fluctuations in plasma glucose level, and play an important role in glucose homeostasis. Recent studies show that gain-of-function and loss-of-function mutations in K$_{ATP}$ channel subunits cause neonatal diabetes mellitus and congenital hyperinsulinism respectively. These findings lead to significant changes in the diagnosis and treatment for neonatal insulin secretion disorders. This review describes the physiological and pathophysiological functions of K$_{ATP}$ channels in glucose homeostasis, their specific roles in neonatal diabetes mellitus and congenital hyperinsulinism, as well as future perspectives of K$_{ATP}$ channels in neonatal diseases.

Keywords: ATP-sensitive potassium (K$_{ATP}$) channels; channelopathy; neonatal diabetes; congenital hyperinsulinism; glucose homeostasis; diazoxide; sulfonylureas

Introduction

Adenosine triphosphate (ATP)-sensitive potassium (K$_{ATP}$) channels function as metabolic sensors that are capable of coupling a cell's metabolic status to electrical activity in order to regulate many cellular functions. The K$_{ATP}$ channels are expressed extensively in various cell types, including pancreatic beta cells, skeletal muscles, smooth muscles, adipose tissue, cardiomyocytes and neurons, where they regulate cell excitability. In pancreatic beta cells, K$_{ATP}$ channels are capable of modulating insulin secretion in response to fluctuations in plasma glucose levels, and thus are an important regulator of glucose homeostasis. K$_{ATP}$ channel mutations mediated dysfunctions are associated with a variety of insulin secretion disorders, including neonatal diabetes mellitus and congenital hyperinsulinism. Fortunately, the identification of the role of K$_{ATP}$ channels in these diseases has led to the implementation of new and improved clinical diagnoses and treatment practices. This review provides an overview of K$_{ATP}$ channels, the role they play in the pathophysiology of neonatal diabetes and congenital hyperinsulinism, and the new therapeutic approaches developed based on our current understanding of

these diseases. It also discusses the current issues associated with the use of K$_{ATP}$ channel modulators in treating these neonatal diseases.

Structure of K$_{ATP}$ channel subunits

Functional K$_{ATP}$ channels are octomeric protein structures composed of four Kir6.x subunits that form the channel pore, surrounded by four sulfonylurea receptors (SURs) that regulate the channel pore activity[1] (Figure 1). The Kir6.x subunits (either Kir6.1 or Kir6.2) belong to the superfamily of weak inwardly rectifying, voltage independent potassium (K$^+$) channels[2]. These channels generate large K$^+$ conductance at potentials negative to the equilibrium potential of potassium (E_K), but permit less current flow at more positive potentials. This, in conjunction with their voltage-independence, makes Kir channels one of the major regulators of resting membrane potentials. Furthermore, the unique sensitivity to ATP/ADP levels makes K$_{ATP}$ channels the ideal cell metabolic sensors[3]. That is, K$_{ATP}$ channels are able to regulate the membrane excitability in response to the metabolic status of the cell, thus, regulating many cellular functions, such as insulin secretion, excitability and cytoprotection.
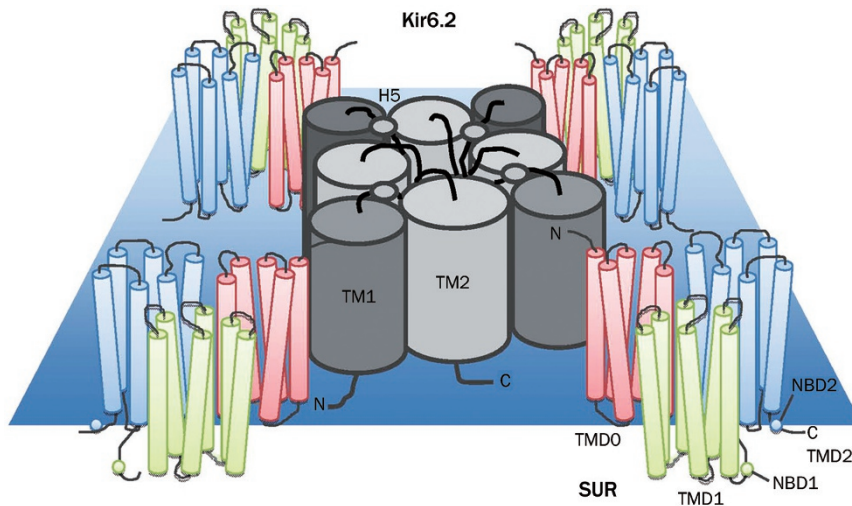
K$_{ATP}$ channels were first discovered in cardiomyocytes[4], and their expression was subsequently confirmed in pancreatic beta cells[5, 6], skeletal muscles[7, 8], vascular smooth muscles[9], adipose tissue[10], glial cells[11], and neurons[12–14]. In the major-

Figure 1. Schematic of the domain structure of a K_{ATP} channel. Four Kir6.2 subunits form the channel pore, and are surrounded by four sulfonylurea receptors (SURs) that regulate pore activity. Each Kir6.2 subunit has two domains (TM1 and TM2), linked by an extracellular pore-forming region (H5). Each SUR subunit has 17 transmembrane regions grouped into three domains: TMD0 (TM1-5), TMD1 (TM 6-11), and TMD2 (TM 12-17). Each SUR subunit also has two nucleotide binding domains (NBD).

ity of these tissues, the channel pore is formed by four Kir6.2 subunits[15]. Each Kir6.2 subunit has two transmembrane (TM) domains (TM1 and TM2) linked by an extracellular pore-forming region (H5)[2, 3]. The four TM2 domains in a K_{ATP} channel form the channel pore and the H5 region serves as the potassium selectivity filter, which contains the Kir6.2 channel signature sequence GFG (rather than GYG in the K^+ channel)[2]. The amino and carboxyl terminals are both found cytoplasmically, and join together to form the cytoplasmic domain that is responsible for channel gating and ATP binding[2, 3, 16]. Like other Kir subunits, it lacks the S4 voltage sensor region that is critical for gating in all voltage dependent calcium (Ca^{2+}), sodium (Na^+) and potassium (K^+) channels. Therefore, K_{ATP} channels are constitutively active if other regulatory mechanisms, such as SUR subunits, or ATP are absent.

The SUR regulatory subunits belong to the ATP-binding cassette (ABC) transporter family[17]. Each SUR subunit has 17 TM regions, grouped into three transmembrane domains (TMDs). TMD1 (TM6-11) and TMD2 (TM12-17) are conserved among members of the ABC family. TMD0 (TM1-5) is unique to SUR, and is essential for trafficking Kir6.2 subunits to the membrane surface[18]. Similar to all other ABC transporters, SUR subunits contain two nucleotide binding domains (NBDs). Dimerization of these two NBDs creates one single nucleotide binding site and catalytic site. Mg-dependent hydrolysis at this site provides the power stroke necessary to overcome the inhibitory effect of ATP on Kir6.2[3]. Unlike traditional ABC transporters, the SUR regulatory subunits cannot conduct a functional current[17].

Two types of SUR regulatory subunits have been identified to date[17, 19]. They differ primarily in their affinity for sulfonylurea, their tissue distribution and genetic source. SUR1, encoded by the *ABCC8* gene located at ch11p15.1, has higher affinity for sulfonylurea and is mainly found in pancreatic beta cells and most neurons. SUR2, encoded by the *ABCC9* gene located at ch12p12.1, has lower affinity for sulfonylureas and is expressed mainly in the heart, skeletal muscle and some neurons[2, 3]. SUR2 has two splice variants, SUR2A and

SUR2B. They differ only in the last 42 C-terminal amino acid residues. Interestingly, the C42 of SUR2B shows high homology to the C42 of SUR1[20]. Since most K_{ATP} channels identified to date contain the Kir6.2 subunit, the heterogeneity observed between different K_{ATP} channels mainly arises from the differential expression of SUR regulatory subunits.

Biophysical properties and regulation of K_{ATP} channels
Gating and channel kinetics
Two independent types of gates for K_{ATP} channels have been described[3, 21, 22]. The fast gating is due to the action of the selectivity filter. This ligand-independent gate is affected by mutations near the selectivity filter[21]. The slow ligand-dependent gate describes changes in the TM2 helices induced by ATP binding[22]. Mutations in TM2 near the cytoplasmic end have been shown to affect the slow gating in multiple Kir channels[3, 23-25]. The kinetics of K_{ATP} channels consists of 1 open state and multiple closed states[3, 21, 26, 27]. The channel only conducts current if all four Kir6.2 subunits are in the open conformation. ATP binding to any one subunit will induce a closed conformation in that subunit, and subsequent channel closure. Therefore the channel has multiple closed states and only one open state.

Trafficking of K_{ATP} channels
The membrane expression of K_{ATP} channels follows the strict 4:4 SUR:Kir stoichiometry, which has two implications. First, it allows only octameric channels expressed on the membrane, thus serves as quality control mechanisms that prevent expression of partial channels. Second, it indicates a tight coupling between the SUR and Kir subunits during the assembly and trafficking of K_{ATP} channels. Indeed, regulation of the membrane expression of the functional channels lies in the presence of a novel ER retention signal, RKR, which is present in both Kir6.2 and SUR subunits[28]. Appropriate interactions between Kir6.2 and SUR subunits result in shielding of this RKR signal, allowing the complex to exit the ER and be trafficked to the plasma membrane. Furthermore, presence of this RKR signal

prevents the membrane expression of Kir tetramers (without SUR), SUR monomers or partial K_{ATP} channel complexes[28]. As such, SUR subunits are the major regulator of the trafficking of Kir6.2 channels.

Numerous factors affect the efficiency of trafficking of K_{ATP} channels. Specifically, SUR regulation of Kir6.2 trafficking is dependent on the TMD0[29] and NBD1[30] domains of SUR, such that mutations (or deletions) at these regions result in significant decreases of membrane expression of K_{ATP} channels. Furthermore, mutations affecting the shielding of the RKR ER retention signal, such as L1544P on SUR1 that causes congenital hyperinsulinism, also decrease trafficking of K_{ATP} channels[31]. Lastly, glucose deprivation stimulates trafficking of Kir6.2 subunits, possibly through an AMP kinase (AMPK)-dependent pathway[32].

Regulation by intracellular ATP, Mg-ADP

The primary regulators of K_{ATP} channel activity are intracellular nucleotides, ATP and ADP. ATP has two major functions[2]. First, it exerts a strong inhibitory effect on K_{ATP} channels via interaction with the cytoplasmic domain of Kir6.2 subunit[33, 34]. Each Kir6.2 subunit has one ATP binding site, so the functional channel can accommodate four ATPs[35]. The ATP binding pocket on each Kir6.2 subunit is formed by residues R50, I182, K185, R201, G334[2, 3, 23, 33, 36–43]. Mutations at any one of these locations can reduce ATP mediated inhibitory effect on Kir6.2, resulting in increased K_{ATP} channel activity. For example, R201H is one of the most common mutations that cause neonatal diabetes[44]. Decreased ATP mediated inhibition results in channel over-activity, and the cell remains hyperpolarized regardless of the ATP level. Other mutations that can alter the effect of ATP also change the intrinsic opening kinetics of the channels[45]. ATP preferentially binds to the closed state of the channel[2–3]. Thus mutations that alter the gating characteristics of the channel, such as I296 in neonatal diabetes, reduce the effectiveness of ATP binding and lead to channel overactivity[45].

ATP, in the presence of Mg^{2+}, also exerts a weak stimulatory effect on K_{ATP} channels via its interaction with SUR regulatory subunits[46]. It is important to note that the principal role of ATP is inhibition. Binding of ATP to any one of the four Kir6.2 subunits will render the channel closed. Under normal physiological ATP levels, the open probability of K_{ATP} channels is less than 0.1% if SUR regulatory subunits are absent[2, 3].

Mg-ADP serves as the principle physiological activator of K_{ATP} channels, and allows them to operate in an ATP insensitive state. Mg-ADP exerts its stimulatory function via interaction with SUR regulatory subunits[2]. Each SUR subunit has two nucleotide binding domains (NBDs), and dimerization of the two NBDs generates the catalytic sites for ATP hydrolysis, and thus dimerization is essential for successful transduction of ADP's stimulatory effect[47–49]. Mutations that disrupt dimerization reduce ADP mediated activation of K_{ATP} channels[50]. Mutations throughout the SUR1 subunit have been identified as one of the major contributors to the occurrence of congenital hyperinsulinemia hypoglycaemia[51]. For exam-

ple, the point mutation G1479R[52, 53] in the NBD2 of SUR1 or V187D[54] in the TMD0 of SUR1, reduced the channel responsiveness to ADP. The decreased ADP-mediated channel activation leads to membrane depolarization in pancreatic beta cells, and continuous release of insulin into the bloodstream, even when plasma glucose levels are low, thus leading to hyperinsulinemic hypoglycaemia.

Regulation by protein interactions

SUR subunits are essential regulatory subunits of K_{ATP} channels. They are necessary for transducing the effect of Mg-ADP, and are the major target site for pharmacological substances. It is unclear how SUR subunits modulate Kir activity; however it has been proposed that the TMD0 domain of SUR anchors SUR to the outer TM1 helix and N-terminus of Kir6.2, thus providing a direct route for information transfer between SUR and the related Kirs[55–57].

K_{ATP} channel activity in pancreatic beta cells and cardiomyocytes can by suppressed by the SNARE protein syntaxin 1A via protein-protein interaction[58]. Two specific mechanisms have been proposed. First, syntaxin 1A interacts with the NBD1 of SUR subunits via its C-terminal H3 domain to decrease the activity of existing plasma membrane K_{ATP} channels when ATP levels are lowered[58–60]. This effect is subject to ATP regulation, such that ATP dose-dependently inhibits syntaxin 1A binding to SUR1 subunits at physiological concentrations[61]. Second, it causes downregulation of K_{ATP} channel expression, either by accelerating endocytosis of existing surface channels, or by decreasing the biogenesis of K_{ATP} channels in the early secretory pathway[62]. Syntaxin 1A binding to SUR1 subunit is able to counter the stimulatory effects exerted by potassium channel openers (KCOs) such as diazoxide, NNC55-0462, P1075 and cromakalim[63, 64]. The physiological role of K_{ATP} channel regulation by syntaxin 1A is presently unclear. However, through modulating K_{ATP} channel activity, syntaxin 1A may play an important role in regulating insulin secretion and in pathologies related to glucose homeostasis.

Other mechanisms of regulation

Another modulator of K_{ATP} channel activity is phosphatidylinositol 4,5-bisphosphate (PIP2). Two possible mechanisms may account for its activating effects[2]. First, it sustains K_{ATP} channel activity by stabilizing the open state, thus decreasing ATP sensitivity and its ability to close the channel. Furthermore, the binding site for PIP2 is also on the cytoplasmic domain of Kir6.2 (R54, R176, 177, R206) and is situated very close to the ATP binding site[65–67]. Thus PIP_2 binding to the channel subunit may allosterically reduce ATP affinity for the channel. Other modulators of K_{ATP} channel activity include protein kinase A (PKA) in smooth muscle cells[68, 69] and cytoskeletal actin in the cardiac atrium[70]. Protein kinase C (PKC) activates the cardiac and pancreatic K_{ATP} channels by phosphorylating T180 at the pore-forming subunit Kir6.2[71]. In the hypothalamus, PKC phosphorylation activates the neuronal Kir6.2/SUR1 K_{ATP} channels to inhibit hepatic glucose production[72]. On the other hand, PKC phosphorylation

stimulates internalization of K_{ATP} channels in cardiomyocytes and CA1 hippocampal neurons, thus functionally decreases K_{ATP} channel activity[73]. Lastly, PKC-mediated activation and upregulation of K_{ATP} channels also play an important role in ischemic preconditioning[74–78].

Pharmacology
Sulfonylureas

Sulfonylureas reduce K_{ATP} channel activity by binding to SUR subunits. They include acetohexamide, tolbutamide, glipzide, glibenclamide and glimepiride. In pancreatic beta cells, the decreased K^+ efflux induced by sulfonylureas leads to membrane depolarization and activation of voltage-gated calcium channels (VGCCs), thus allowing Ca^{2+} influx and insulin release. As such, sulfonylureas have been used to treat diabetes and related diseases. Sulfonylureas therapy is one of the most established treatments for type 2 diabetes, as it is very effective and cost-efficient in achieving the targeted glycemic goals[79]. Its major advantage is its rapid effectiveness, and its major side effects include hypoglycaemia and weight gain. With the discovery of the role of Kir6.2 mutations in causing neonatal diabetes mellitus, sulfonylureas have also become the main drug used to treat this disease. Patients achieve better glycemic control with sulfonylureas compared to insulin injections, and many of the side effects observed in type 2 diabetics (*eg*, hypoglycaemia) are not seen in patients with neonatal diabetes[80].

Modulation of sulfonylureas

The effect of sulfonylureas is altered by the presence of cytoplasmic nucleotides such as Mg-ADP. It has been shown that in the cell-attached configuration, sulfonylureas can completely block K_{ATP} channels[51]. In excised membrane patches however, the blockage is only 50%–70%. This difference is attributed to the presence of Mg-ADP, which can strongly activate K_{ATP} channels via its interaction with SUR1 and weakly inhibit K_{ATP} channel activity through the ATP binding site on Kir6.2. The strong stimulatory effect of Mg-ADP, but not the weak inhibitory effect, is counteracted by sulfonylureas. Therefore, the presence of Mg-ADP appears to enhance sulfonylureas' inhibitory effects[51, 81].

Binding sites for tolbutamide and glibenclamide have been described. Both drugs share the high affinity binding site S1237 located in cytoplasmic loop 8 between TM15 and TM16 of SUR[82, 83]. The other, low affinity binding site for tolbutamide is located on Kir6.2[84, 85]. Another binding site for glibenclamide is located in cytoplasmic loop 3 between TM5 and TM6[82, 83].

Potassium channel openers

The other class of drugs are K channel openers (KCOs) and these include cromakalim, pinacidil, nicorandil, diazoxide and minoxidil sulphate. Like their name suggests, these drugs bind to SUR regulatory subunits to stimulate K_{ATP} channel activity[2]. In pancreatic beta cells, diazoxide binds to SUR1 subunits to increase K_{ATP} channel activity, promoting K^+ efflux

and cell hyperpolarization. This reduces the amount of insulin released. As such, this drug is currently one of the major drugs used to treat congenital hyperinsulinism[86, 87].

Different K_{ATP} channels respond differently to these KCOs due to expression of different SUR regulatory subunits. Pancreatic beta cell K_{ATP} channels are composed of Kir6.2 and SUR1 subunits, and are readily activated by diazoxide, weakly activated by pinacidil, and unaffected by nicorandil or cromakalim[88]. In contrast, cardiac K_{ATP} channels, which are composed of Kir6.2 and SUR2A subunits, are activated by pinacidil, nicorandil and cromakalim, but not affected by diazoxide[89, 90]. Interestingly, K_{ATP} channels in smooth muscles respond to all of these drugs[91]. The observed differences in KCO sensitivity is due to differences in SUR subunits. SUR1 shows high sensitivity to diazoxide, and SUR2A shows high sensitivity to pinacidil, nicorandil and cromakalim. The binding site for cromakalim, pinacidil and nicorandil resides within the second TM domain of SUR. The nucleotides L1249 and T1253 in SUR2A, and T1286 and M1290 in SUR2B are necessary and sufficient for KCO binding[92–94]. These differences underscore the importance of SUR subunits in determining the function of K_{ATP} channels.

Physiological role of K_{ATP} channels in glucose homeostasis

K_{ATP} channels across several different tissues contribute to glucose homeostasis. In pancreatic beta cells, K_{ATP} channels consisting of Kir6.2 and SUR1 subunits[95] promote insulin secretion and thus cause a reduction in blood glucose concentration[96] (Figure 2). In their open state, K_{ATP} channels permit an efflux of K^+ ions to maintain the cell's polarized membrane potential. Following the metabolism of glucose, however, it is believed that the ATP that is produced causes K_{ATP} channels to close and thus no longer contribute to the polarization of the cell. The cell is now more depolarized and this triggers the influx of calcium via VGCCs and consequently the release of insulin-containing granules[96].

K_{ATP} channels also play a role in regulating the release of glucagon. Insulin and zinc ions activate K_{ATP} channels on pancreatic alpha cells to hyperpolarize them and thus inhibit their ability to release glucagon[97]. Alternatively, glucose can act to inhibit K_{ATP} channels in pancreatic alpha cells[98]. However, unlike in beta cells where the inhibition of K_{ATP} channels is excitatory, the inhibition of K_{ATP} channels in alpha cells is inhibitory and therefore inhibits the release of glucagon[96, 99]. This is because in alpha cells, the resting membrane potential is much closer to the threshold potential for action potential firing than in beta cells. Therefore, the depolarization resulting from K_{ATP} channel closure is sufficient to maintain VGSCs in a state where they are unable to reconfigure - thus inducing a depolarization block. In this way, the inactivation of K_{ATP} channels is excitatory in beta cells, but inhibitory in alpha cells.

K_{ATP} channels may regulate glucose concentration by mediating glucagon release at the level of the hypothalamus. Kir6.2 subunits are required for the increased spontaneous discharge rate of neurons in the ventromedial hypothalamus (VMH)
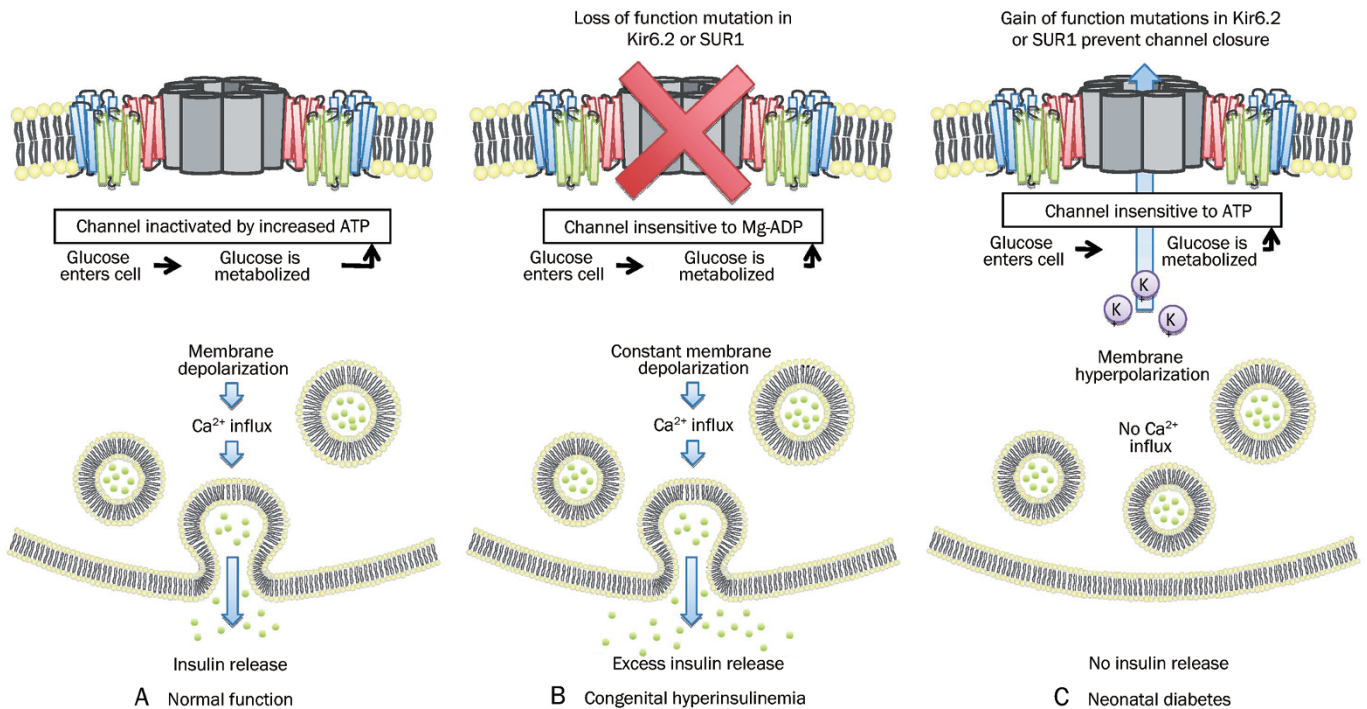
Figure 2. The role of K_{ATP} channels in insulin release from pancreatic β-cells. When glucose enters the cell it is metabolized to yield ATP. (A) During normal function, the K_{ATP} channel is inactivated, causing membrane depolarization and insulin release. (B) Loss of function mutations in Kir6.2 or SUR1 result in constant membrane depolarization leading to the excess insulin release characteristic of congenital hyperinsulinemia. (C) Gain of function mutations in Kir6.2 or SUR1 promotes the open state of the channel to cause membrane hyperpolarization and the lack of insulin release characteristic of neonatal diabetes.

following an increase in the concentration of extracellular glucose[96]. This increase in VMH activity is likely a reflection of increased firing of glucose-responsive neurons (GRNs) within the VMH[96, 100]. In addition, neurons within the VMH have been implicated in an autonomic pathway involving the release of adrenaline and terminating with the release of glucagon from pancreatic alpha cells[99, 101]. VMH K_{ATP} channels are composed of Kir6.2 and SUR1 subunits, just like in pancreatic beta cells[102]. Therefore, the ability for the body to regulate blood glucose levels by releasing both insulin and glucagon likely depends on these specific K_{ATP} channels in pancreatic beta cells and VMH cells, respectively. K_{ATP} channels in the hypothalamus may modulate the production of glucose[103]. The infusion of the K^+ channel opener diazoxide into the hypothalamus inhibits gluconeogenesis in the liver, and a global knockout of SUR1 prevents the inhibition of gluconeogenesis by insulin[103]. Insulin acts on K_{ATP} channels via the phosphatidylinositol 3-kinase (PI3K)/phosphatidylinositol 3,4,5-triphosphate (PIP$_3$) pathway in the arcuate nucleus of the hypothalamus to inhibit the release of glucose from the liver[104]. Pro-opiomelanocortin-expressing neurons originating from this nucleus are sensitive to K_{ATP} channel activation and partial activation of these neurons results in impaired glucose tolerance[105].

Finally, K_{ATP} channels may regulate glucose concentration by mediating the uptake of glucose into skeletal muscles.

The knockout of Kir6.2 subunits is associated with increased absorption of glucose into skeletal muscle (both basally and in response to insulin)[106], as well as an increased sensitivity of blood glucose concentration to insulin[102]. Similar effects are evident following the knockout of the SUR2 regulatory domain. Therefore, this K_{ATP} channel likely acts to inhibit glucose uptake in its open state and promote glucose uptake in its closed state[107, 108]. Although the mechanism of such action is unknown, evidence suggests that this uptake of glucose is independent of the insulin receptor substrate-1/PI3K signalling pathway that underlies the sensitivity of skeletal muscles to insulin[109–111]. It is also believed to be independent of the insulin-independent cAMP-activated protein kinase dependent pathway[112, 113]. Taken together, K_{ATP} channels, acting through different mechanisms and from within various tissues, contribute to the regulation of blood glucose levels, and regulate glucose homeostasis under both physiological and pathological conditions.

Pathophysiological role of K_{ATP} channels in glucose homeostasis
Mutations in K_{ATP} channel subunits and neonatal diabetes mellitus
Neonatal diabetes mellitus (NDM) is defined as the occurrence of insulin-requiring monogenic diabetes in the first six months of life[44, 114, 115]. It is a rare disease with incidence in the

range of 1/200 000 live births[115–118]. It can be transient, which is characterized by spontaneous remission within the first few months of life, or permanent, in which continuous treatment is required from the time of diagnosis. Transient neonatal diabetes mellitus (TNDM) is a result of chromosome abnormalities in the 6q24 locus[80]. The PLAGL1/ZAC gene, a zinc finger protein that regulates apoptosis and cell cycle arrest and the HYMAI gene are believed to be involved in TNDM, although the mechanism of action is poorly understood[80, 119, 120]. In contrast, permanent neonatal diabetes mellitus (PNDM) most commonly results from activating mutations in the genes encoding Kir6.2 (*KCNJ11*) and its regulatory subunit SUR1 (*ABCC8*)[44]. Mutations in other genes, including insulin[121–126], insulin promoter factor[127–129], glucokinase[130–139] and FOXP3[140, 141] have also been reported to cause PNDM. Understanding the genetic basis of NDM in general has greatly facilitated the correct diagnosis and treatment of this disease. The following sections will focus on the role of K_{ATP} channel subunits in PNDM, and the current therapeutic approaches[80].

Extensive clinical and molecular studies have firmly established the role of K_{ATP} channel subunits, specifically, Kir6.2 and SUR1 in neonatal diabetes. Overexpression of mutant Kir6.2 subunits with reduced ATP sensitivity causes mice to develop severe neonatal diabetes[142]. More importantly, a polymorphism in Kir6.2 (E23K) is consistently linked with adult diabetes mellitus[143–148]. The role of Kir6.2 in PNDM was confirmed in 2004, when Gloyn *et al* reported that activating dominantly inherited mutations in *KCNJ11* were found in 10 out of 29 patients with NDM[44]. These patients did not secrete insulin in response to glucose or glucagon, but did secrete insulin in response to tolbutamide, which is a K_{ATP} channel blocker used to treat type 2 diabetes. Six heterozygous mutations of Kir6.2 were identified, among which the mutations R201H and V59M were most common. When the R201H mutant was co-expressed with SUR1 in *Xenopus* oocytes, the resulting mutant channels showed 40× decreased sensitivity to ATP inactivation compared to the wild type channels[44].

Recent studies have identified more than 40 mutations in Kir6.2 and a similar number in SUR1 that lead to PNDM[149]. All Kir6.2 mutations are heterozygous (dominantly inherited), but SUR1 mutations are more heterogeneous, with homozygous, heterozygous and compound heterozygous mutations being described[80, 149]. About 80% of *KCNJ11* mutations and 50% of *ABCC8* mutations arise *de novo*[150], however, there were two reports of germline mosaicism where two siblings with PNDM were born to unaffected parents[151]. Interestingly, Kir6.2[152–159] and SUR1[160–166] mutations have also been found in TNDM patients, who do not have mutations at the 6q24 locus.

All mutations reported to date are missense mutations, with the exception of one in-frame KCNJ11 nucleotide deletion[167]. The functional consequence of Kir6.2 and SUR1 mutations is reduced metabolic sensing capacity of the K_{ATP} channel. All mutations on Kir6.2 decrease the channel's sensitivity to ATP[2, 134, 168], either directly by interfering with ATP binding[37, 44, 159, 169, 170], or indirectly by decreasing the intrinsic

open probability of the channel[45, 170]. Mutations that affect ATP binding directly are clustered near the binding site, and these include the common R201H mutation, and also R50, I182, Y330, and F333[2, 3, 44]. The second, indirect mechanism by which Kir6.2 mutations increase channel function lies in the fact that ATP sensitivity is decreased when the channel is in the open state. Thus mutations that drive the channel to the open state, such as I196H located at the channel pore, can decrease the ability of ATP to close the channel[45]. Mutations in SUR1 function mainly increase the Mg-nucleotide mediated activation of the channel or change the intrinsic gating properties of the channel[2, 3]. Overall, these mutations result in the gain of function of K_{ATP} channels so that they are persistently open, leading to beta cell hyperpolarization even in the presence of elevated plasma glucose levels. Hyperpolarization prevents the secretion of insulin, thus resulting in the diabetic phenotype.

Mutations in K_{ATP} channel subunits and DEND syndrome

About 20% of patients with PNDM exhibit developmental delay, epilepsy, muscle weakness in addition to neonatal diabetes (DEND syndrome)[171]. Patients with a milder form, termed intermediate DEND (iDEND), do not have epilepsy. There are fifteen Kir6.2 mutations and two SUR1 mutations that may cause DEND and iDEND[149–168]. In particular, the V59M mutation in Kir6.2 is the most common cause of iDEND[44, 142, 168, 172]. The genetic basis of DEND suggests that mutation of this channel is responsible for all of the observed symptoms, and this notion is strengthened by the fact that K_{ATP} channel subunits are expressed in the affected tissues, namely the brain, muscle and pancreas.

The diabetic phenotype is the result of K_{ATP} channel overactivity in pancreatic beta cells, the same as that observed in PNDM. The muscle dysfunction is neural in origin[172]. Several lines of evidence illustrate this. Muscle weakness is observed in one patient with a SUR1 mutation, despite the lack of SUR1 expression in skeletal muscles[173]. Also, muscle weakness and ataxic gait in a patient with a Kir6.2 mutation was improved by treatment with gliclazide, which interacts only with SUR1[174]. More definitive evidence comes from a recent study by Clark *et al*, in which hemizygous mice that selectively express the V59M Kir6.2 mutation in muscle or neurons were examined[172]. Behaviourally, transgenic mice with the neuronal V59M Kir6.2 mutation display the same motor impairments as seen in human DEND. Electrophysiological studies show that V59M-carrying Purkinje neurons (output of cerebellum that regulates motor movement) were hyperpolarized and displayed suppressed electrical firing. This was reversed with the application of tolbutamide, suggesting that neuronal K_{ATP} channels containing V59M Kir6.2 were overactive mutants. In contrast, muscle-specific V59M mutation failed to alter muscle membrane properties, and mice with muscular V59M Kir6.2 mutation behave like their wild type counterparts. The differential effects of Kir6.2 V59M mutation on neurons and muscles may be due to the divergence in the identity of SUR regulatory subunits. Neurons, like pancreatic beta-cells, mostly contain

K_{ATP} channels made up of Kir6.2 and SUR1 subunits, whereas muscle K_{ATP} channels are composed of Kir6.2 and SUR2A subunits. In fact, it has been shown that the Kir6.2 V59M mutation specifically enhances the flow of current through K_{ATP} channels composed of Kir6.2/SUR1 subunits, but has no effect on Kir6.2/SUR2A K_{ATP} channels[172]. Thus, the V59M mutation selectively targets the pancreas and neurons while sparing the muscle.

The last two symptoms of DEND, namely developmental delay and epilepsy, are neuronal in origin, but their exact causes are unknown. It has been proposed that epilepsy may result from decreased activity of inhibitory interneurons in the hippocampus, as there's a greater density of K_{ATP} channels in the inhibitory neurons compared to the excitatory ones[11, 173]. The cause of developmental delay is unclear, but it may result directly from overactivity of K_{ATP} channels, or may be secondary to the symptom of diabetes. Motor and cognitive development requires dynamic changes in neuronal networks and balanced excitatory and inhibitory inputs within the network. Overactivity of K_{ATP} channels hinders the occurrence of excitatory synaptic connections, and thus may inhibit neuronal activity and development. On the other hand, developmental delay may be the result of diabetes-induced neuropathy. Therefore, further research is warranted to elucidate this matter.

Therapeutics of neonatal diabetes
Neonatal diabetes was originally thought to be an early onset form of type 1 diabetes and was therefore treated with insulin injections[80]. However, after the discovery of the mutations of K_{ATP} channel subunits, more than 90% of patients were switched to treatments with sulfonylureas (0.05–1.5 mg·kg^{-1}·d^{-1})[175]. Most patients exhibited significant improvement in their clinical situations[168]. Specifically, blood glucose levels were generally reduced, as indicated by HbA1C levels[175–179]. There were also less fluctuation in plasma glucose levels, and hypoglycaemic episodes were less common[173]. In addition, patients with iDEND also see improvement in extrapancreatic symptoms, such as reduced epileptic events, improved cognition and improved muscle tone and balance[173, 180–183]. Unfortunately, patients suffering from the severe DEND syndrome are less responsive to sulfonylurea treatment[184–186]. Side effects are minimal; a few patients have reported transient diarrhoea and tooth discoloration[173, 187, 188].

Sulfonylureas work by interacting with SUR subunits to induce channel closure. This allows for the depolarization of the cell membrane and increased excitability. In pancreatic beta cells, cell depolarization allows for the activation of voltage-gated calcium channels, stimulating an influx of Ca^{2+} into the cytoplasm and subsequent release of insulin into the bloodstream. This is able to mitigate the diabetic symptoms. Interestingly, there was better insulin response to oral glucose intake than to intravenous glucose in NDM patients treated with sulphonylureas[175–189]. The presence of food in the gut lumen stimulates the secretion of hormones and signalling molecules such as GLP-1, GIP and ACh, which can amplify the

insulin response and it appears that their proper action may require K_{ATP} channel closure[189]. How sulphonylureas affect the secretion of such hormones is unclear at this moment. It has been proposed that secretion of these hormones requires Ca^{2+} influx, which in patients with PNDM is only possible after sulfonylurea treatment[189].

The improvement of neurological symptoms of DEND and iDEND is most likely due to the action of sulfonylureas on neuronal K_{ATP} channels. Although there is enhanced cognition, improved muscle tone and abolition of seizures, development does not return to normal with sulfonylurea treatment[173]. This could be the result of insufficient sulfonylurea potency or the irreversibility of neuronal damage caused by K_{ATP} channel overactivity.

A point of concern is that glibenclamides and glyburides, which interact with both SUR1 and SUR2A, are currently used to treat DEND and iDEND. The discovery of the neuronal origin of muscle impairment in DEND and iDEND suggests that more specific drugs, that only target SUR1, could be used in order to avoid unnecessary side effects, especially in the myocardium which also express SUR2A subunits[172]. Indeed, it has been reported that gliclazide, which only interacts with SUR1, can alleviate the motor symptoms[174].

Another minor drawback with sulfonylurea therapy is that its effectiveness decreases as the time between diagnosis and transfer to sulfonylurea therapy increases, although it is relatively successful when used at the early stage of the disease[80, 175]. Prolonged hypoinsulinemia may result in a loss of beta cells and thus the insulin secreting machinery downstream of K_{ATP} channels may not be functional[173]. In mouse models of neonatal diabetes, prolonged lack of insulin leads to a progressive decrease in beta cell mass and a loss of normal islet architecture is observed[190]. In such cases, even though sulfonylurea may enhance K_{ATP} channel closure, it cannot relieve the hyperglycemia. Thus, it is beneficial to start sulfonylurea treatment as early as possible if neonatal diabetes is diagnosed. However, sulfonylurea treatment is only effective for those with mutations in K_{ATP} channel subunits, which only account for half of all NDM patients. For patients with mutations in other genes such as insulin and glucokinase, it may not be beneficial. Therefore, it may be advantageous for all diabetic patients to undergo genetic testing in the first six months of life in order to obtain a definitive diagnosis, and permit the earliest possible commencement of treatment[80].

K_{ATP} channels and congenital hyperinsulinism
Congenital hyperinsulinism (CHI) is characterized by continuous and unregulated insulin secretion despite low plasma glucose levels[51, 191, 192]. The incidence of CHI is 1/30 000 to 1/50 000 live births per year, however in some isolated areas where inbreeding is common, the disease incidence may reach 1/2500[193]. This disease cannot be detected in utero, and babies with CHI have no gross characteristic differences from normal babies[51]. The first clinical signs are vague, and include cyanosis, respiratory distress, sweating, hypothermia, poor feeding and hunger. It is important that the correct diagnosis is made

promptly, as delayed treatment will result in permanent brain damage and mental retardation due to insufficient energy supply for brain metabolism[51].

There are two distinct forms of CHI, categorized based on their histological differences. Focal CHI is characterized by the presence of a small endocrine lesion in the pancreas. In the lesion area, the islets are normally structured, but hyperplastic; outside of the legion, the islets are normal[194–196]. Complete resection of the lesion can cure the patient. On the other hand, in patients with diffuse CHI, the islets of Langerhans show large beta cells with abnormally large nuclei, which is indicative of hyperactivity. Treatment for diffuse CHI patients usually involves partial pancreatectomy[196], which often leads to pancreatic insufficiency and iatrogenic diabetes mellitus[51].

Mutations in K_{ATP} channel subunits are the most common cause of CHI[51]. The SUR1 gene *ABCC8* and Kir6.2 gene *KCNJ11* are located at ch11p15. Mutations at this genetic locus are linked to both diffuse and focal CHI[197]. Diffuse CHI predominantly arises from autosomal recessive mutations of K_{ATP} channel subunits, although dominant ones have been reported[51–193]. Focal CHI results from loss of heterozygosity at this locus[198–204]. In the normal part of the pancreas, the cells inherit a mutated SUR1 gene from the father and a normal SUR1 gene from the mother. These cells have a normal phenotype, due to expression of the normal maternal SUR1 gene. In the focal lesion, the cells have lost the normal maternal chromosome during fetal life and contain two copies of the mutated SUR1 genes inherited from the father. They have also lost the maternally imprinted tumor suppressor genes P57[kip2], although they still express the paternally derived insulin-like growth factor II gene. This combination enables the growth of the focal lesion. About 40%–65% of patients with CHI have focal CHI[51].

Mutations of K_{ATP} channels responsible for CHI are mostly found in SUR subunits, and a few in Kir6.2 subunits[51, 168–205]. Indeed, over 150 mutations have been identified in the SUR1 subunit and these account for 50% of all CHI cases[149]. These loss-of-function mutations have been grouped into two classes[168]. In class I, there is a total loss of K_{ATP} channels in the plasma membrane, resulting in no K_{ATP} current[51]. This type of mutation accounts for 10% of all diffuse CHI patients and 55% of focal CHI patients. The most common cause is defects in trafficking[168]. For example, the mutation R1437Q(23)X in exon35 of *ABCC8* causes truncation of the C-terminus of SUR1, which contains the signal sequence necessary for exiting the ER[51, 206]. Thus, the channel protein is retained in the ER and cannot be expressed in the membrane. In class II mutations, K_{ATP} channels are present in the membrane (although less than normal) but show reduced sensitivity to Mg-nucleotide activation or reduced intrinsic channel open probability[51, 168]. These mutations account for more than 60% of diffuse CHI cases and 45% of focal CHI cases. For example, point mutations such as G1479R in NBD2 of SUR1[52] or V187D in the TMD0 of SUR1[207] lead to reduced responsiveness to ADP activation in the expressed channels. Overall, these mutations result in a loss-of-function of K_{ATP} channels in the pancreatic beta cells, leading to constitutive exocytosis of insulin-containing secretory vesicles. In addition to K_{ATP} channel related mutations, CHI also arises from autosomal dominant mutations in genes involved in glucose metabolism, including mutations in glutamate dehydrogenase (GDH)[208–216], glucokinase[217, 218] and short-chain L-3-hydroxyacyl-CoA dehydrogenase (SCHAD)[106–219].

Therapeutics of congenital hyperinsulinism

Understanding the molecular mechanisms that underlie CHI provides the basis for establishing effective treatment protocols. Of the utmost importance is the correct diagnosis of the type of CHI. Following the identification of K_{ATP} channels in the pathology of CHI, the K^+ channel opener, diazoxide, has become a diagnostic tool and a treatment method[86, 87]. When CHI is suspected, the first step in diagnosis is to determine the diazoxide responsiveness. The diazoxide-responsive CHI patients can be managed with diazoxide treatment with regular monitoring. Non-responsive patients are given a genetic test of the *ABCC8* and *KCNJ11* genes to determine whether it is a case of focal or diffuse CHI. For the diffuse CHI patients, treatment involves a high calorie diet, somatostatin (Octreotide) therapy and a near-total pancreatectomy. Regular follow-up is required to monitor growth, development, as well as the occurrence of diabetes mellitus later in life. For focal CHI patients, a complete resection of the focal lesion is commonly used and can cure patients[86, 87].

The mainstream drug used in CHI treatment, diazoxide (10-20 mg·kg^{-1}·d^{-1}), is a K_{ATP} channel opener and binds to SUR1 subunits to promote K_{ATP} channel opening[51]. This prevents the depolarization of pancreatic beta cells and insulin secretion. Diazoxide is easily administered orally and provides significant relief for CHI caused by mutations in GDH, glucokinase and SCHAD. However, it is not effective for type I K_{ATP}-related CHI, in which a lack of K_{ATP} channels has been found. Therefore, diazoxide has no targets to exert its effects. More potent diazoxide analogues such as HEI713, BPDZ73, BPDZ44, and BPDZ154 have been synthesized[51]. Although effective in stimulating K_{ATP} channel opening *in vitro*, their clinical potential has not yet been determined.

Diazoxide also poses serious side effects. It causes sodium/water retention that can lead to complications such as congestive heart defects, poor cardiac reserve, hyperuricemia and hypotension in patients with heart problems. Moreover, diazoxide can decrease immune function and long term use has been linked with hyperosmolar nonketotic coma[51].

Other drugs used for CHI treatment include L-type Ca^{2+} channel antagonists (nifedipine, verapamil), glucagon, somatostatin and corticosteroids[51]. VGCC antagonists prevent Ca^{2+} influx and may decrease insulin secretion. They have been shown to be therapeutically beneficial in some[220–223], but not all CHI patients[224]. Glucagon is used because it stimulates glycogenolysis and gluconeogenesis, but it is disfavoured because it also acts as an insulin secretagogue, thus promoting insulin hypersecretion. Long-term use of somatostatin is widely accepted, as it potently inhibits insulin release via acti-

vation of hyperpolarizing K$^+$ channels and it independently inhibits VGCCs. Short term use of steroids helps to maintain adequate blood glucose levels[51].

Perspectives/future directions
Neonatal diabetes mellitus
The identification of the role of K$_{ATP}$ channels in neonatal diabetes has revolutionized the treatment for this disease. With the groundbreaking report in 2004 by Gloyn *et al* that identified mutations in Kir6.2 subunits as one of the major causes of neonatal diabetes[44], more than 90% of patients have been switched to treatment with sulfonylureas, which induce K$_{ATP}$ channel closure. Sulfonylureas provide better glucose control than previous treatment methods, and to date, the side effects reported (*eg*, tooth discoloration) have been minimal. Hypoglycemic episodes, which is the major side effect associated with sulfonylurea treatment in type 2 diabetes, are not observed in patients with neonatal diabetes. However, long-term monitoring is needed to determine whether the other side effects previously reported with sulfonylurea use, such as liver dysfunction, skin allergic reactions, pancytopenia, hyponatremia or cardiovascular abnormalities will appear in patients with NDM. One possible way to minimize potential side effect is to develop derivatives of sulfonylureas specific to pancreatic cells, thus minimizing the unwanted effect on other tissues.

DEND and iDEND
For patients with DEND or iDEND, sulfonylurea treatment not only provides better glucose control, but also alleviates some of the extrapancreatic symptoms. As such, sulfonylurea therapy reduces occurrences of epileptic events, and improves muscle and cognitive functions[173]. However, sulfonylureas do not improve developmental delay. The main difficulty is that the cause of developmental delay is unclear. It could arise from prolonged over-activity of neuronal K$_{ATP}$ channels, or could be secondary to sustained high glucose levels prior to treatment. It is unclear how well sulfonylureas cross the blood brain barrier, thus the potency and efficacy of these drugs on neurons is unknown. It has been suggested that higher potency drugs may better improve the neural symptoms.

Another interesting point is that there are no cardiac symptoms observed in DEND or iDEND, even though K$_{ATP}$ channels are widely expressed in cardiomyocytes. Pancreatic beta cells and neurons express Kir6.2 and SUR1 subunits while skeletal muscle and cardiomyocytes express SUR2A and Kir6.2 subunits. The study by Clark *et al* (2010) demonstrating the neural origin of motor symptoms suggest that skeletal muscle K$_{ATP}$ channels, which are composed of SUR2A and Kir6.2 subunits, are not affected, thus, it is not surprising that DEND or iDEND patients have no cardiac symptoms[172].

This differential response between K$_{ATP}$ channels to the same genetic mutation has several implications. For one, it indicates that more specific sulfonylureas — ones that selectively target SUR1 — should be preferred over those that bind to both SUR2A and SUR1 when treating DEND/iDEND. Next,

it underlines the importance of neuronal K$_{ATP}$ channels in DEND and iDEND. This area is relatively unexplored at this moment. Understanding the effect of these mutations on neuronal networks would provide a better idea about the molecular basis of the observed symptoms, and allow development of better therapeutic approaches. Lastly, it highlights the importance of SUR subunit regulation in K$_{ATP}$ channel activity. Mutations implicated in DEND and iDEND are mostly found in the Kir6.2 subunit, and these subunits are expressed in most tissues. The expression of SUR subunits, however, is tissue-specific and thus SUR subunits have a better capability of regulating mutant channels.

Congenital hyperinsulinism
Identification of the role of K$_{ATP}$ channels has improved the diagnostic and treatment process for many patients suffering with congenital hyperinsulinism (CHI). Since congenital CHI usually results from the loss of K$_{ATP}$ function, K$_{ATP}$ channel openers such as diazoxide are used to counteract the deficit in K$_{ATP}$ function. In cases where K$_{ATP}$ channels are not trafficked to membrane, diazoxide treatment may be ineffective. Nonetheless, diazoxide is useful in two ways. One, it serves as a diagnostic tool for determining the specific type of CHI. Patients in which diazoxide treatment is effective likely do not have mutations in K$_{ATP}$ channel subunits and thus diazoxide can be administered as the key drug for these patients. Since diazoxide is not very effective for CHI patients with K$_{ATP}$ channel mutations, alternative approaches for treating these patients may be to examine novel regulators of K$_{ATP}$ channels. One such molecule is syntaxin 1A, which inhibits K$_{ATP}$ channels[61]. Therefore, decreases in syntaxin 1A levels can increase K$_{ATP}$ channel activity. In contrast to diazoxide which only increases the activity of existing K$_{ATP}$ channels, syntaxin 1A has been shown to regulate the membrane expression of wild-type K$_{ATP}$ channels. Thus, it would be worthwhile to examine whether syntaxin 1A can provide additional regulation and therefore be effective for treating congenital hyperinsulinism.

Conclusion
Understanding the genetic basis of neonatal diabetes and congenital hyperinsulinism has significantly improved the diagnosis and treatment of patients with these diseases. The current use of K$_{ATP}$ channel modulators by these patients has greatly alleviated their symptoms and improved their quality of life. However, assessment of the long-term effects of these treatment methods is warranted and better optimization of the treatment protocol is needed in order to deliver the best possible care to patients.

Acknowledgements

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