Nucleotide Sensitivity of Pancreatic ATP-Sensitive Potassium Channels and Type 2 Diabetes

Christina Schwanstecher and Mathias Schwanstecher

Type 2 diabetes is generally perceived as a polygenic disorder, with disease development being influenced by both hereditary and environmental factors. However, despite intensive investigations, little progress has been made in identifying the genes that impart susceptibility to the common late-onset forms of the disease. E23K, a common single nucleotide polymorphism in K<sub>IR6.2</sub>, the pore-forming subunit of pancreatic β-cell ATP-sensitive K<sup>+</sup> (K<sub>ATP</sub>) channels, significantly enhances the spontaneous open probability of these channels, and thus modulates sensitivities toward inhibitory and activatory adenine nucleotides. Based on previous association studies, we present evidence that with an estimated attributable proportion of 15% in Caucasians, E23K in K<sub>IR6.2</sub> appears to be the most important genetic risk factor for type 2 diabetes yet identified. Diabetes 51 (Suppl. 3):S358–S362, 2002

ROLE OF K<sub>ATP</sub> CHANNELS IN INSULIN SECRETION

Plasma insulin concentrations are normally determined by a feedback system that is controlled mainly by the level of plasma glucose (1). The overall activity of the pancreatic β-cell is set by the sensitivity of peripheral tissues to the action of insulin, with insulin-resistant subjects having increased plasma insulin levels and secretion rates. Insulin secretion is also elicited in response to amino and fatty acids; the extent of this response is modified by neural (e.g., autonomic tone) and hormonal (e.g., glucagon, glucagon-like peptide) factors. Glucose, however, is the dominating factor in controlling insulin secretion.

Glucose enters the β-cell by facilitated diffusion, and its phosphorylation by glucokinase to glucose-6-phosphate determines the rate of glycolysis and the rate of pyruvate generation (Fig. 1) (2,3). Thus the rate of glycolysis will increase with blood glucose. In β-cells, pyruvate is the main product of glycolysis (4) and, compared to other cell types, an unusually high proportion of glucose-derived pyruvate enters the mitochondrial tricarboxylic acid (TCA) cycle (2).

Subsequent oxidative metabolism generates the trigger for insulin secretion (5). Electron transfer from the TCA cycle to the respiratory chain by NADH and reduced flavin adenine dinucleotide (FADH<sub>2</sub>) initiates the production of ATP, which is delivered to the cytosol. Here the rise of the ATP-to-ADP ratio causes a reduction in plasma membrane K<sup>+</sup> conductance, resulting in depolarization of the membrane (6). Hence voltage-sensitive Ca<sup>2+</sup> channels are opened that are similar to those expressed in other excitable cells. This is the critical step by which glucose stimulates insulin secretion, as the increase in cytosolic Ca<sup>2+</sup> is the main trigger for exocytosis (6,7).

The decrease in K<sup>+</sup> conductance results from closure of the ATP-sensitive potassium (K<sub>ATP</sub>) channels (6). These channels dominate the resting membrane potential in β-cells and act as transducers of glucose-induced metabolic changes into electrical activity. Their central role in the stimulation of insulin secretion can easily be demonstrated using channel modulators (e.g., tolbutamide, diazoxide) that do not interfere with glucose metabolism (8).

The significance of membrane-potential control is illustrated by the syndrome of persistent hyperinsulinemic hypoglycemia of infancy (PHHI). PHHI is most frequently caused by mutations in one of the two subunits of the K<sub>ATP</sub> channel, the regulatory sulfonylurea receptor subunit 1 (SUR1) or the inwardly rectifying potassium channel subunit (K<sub>IR6.2</sub>), resulting in permanent depolarization of the β-cell and uncontrolled hypersecretion of insulin (9). However, most PHHI patients show discrete glucose-induced insulin secretion above the constitutively elevated basal rate (10). This observation argues in favor of K<sub>ATP</sub> channel–independent effects of glucose, which is capable of stimulating a partial secretory response under conditions of clamped, elevated cytosolic Ca<sup>2+</sup> (8). Thus closure of K<sub>ATP</sub> channels by ATP generated in the mitochondria appears to be the most critical step in insulin secretion, with other metabolic factors enhancing the secretory response (11).

ROLE OF CYTOSOLIC NUCLEOTIDES IN K<sub>ATP</sub> CHANNEL CONTROL

K<sub>ATP</sub> channels are assembled with a tetrmeric stoichiometry from two structurally distinct subunits: K<sub>IR6.2</sub>, which forms the pore, and SUR1 (12,13). While hypoglycemic sulfonylureas (e.g., glibenclamide, tolbutamide) and potassium channel openers (e.g., diazoxide) exert their effects on channel activity by interaction with SUR1, there

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Emax, maximal effect; f<sub>K</sub>, frequency of the allele with K in position 23 of K<sub>IR6.2</sub> in Caucasians; IC<sub>50</sub>, half-maximal inhibitory concentration value; K<sub>ATP</sub> channel, ATP-sensitive potassium channel; K<sub>IR6.2</sub>, inwardly rectifying potassium channel subunit; OR, odds ratio; PHHI, persistent hyperinsulinemic hypoglycemia of infancy; P<sub>open</sub>, open probability; PPARG-γ, peroxisome proliferator–activated receptor-γ; SNP, single nucleotide polymorphism; SUR1, regulatory sulfonylurea receptor subunit 1; TCA, tricarboxylic acid.

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is strong evidence that the receptor site for inhibitory ATP is formed by Kir6.2.

The defining characteristic of the K_ATP channel is that its activity is inhibited by an increase in the intracellular ATP concentration. When measured in inside-out patches, the channel is highly ATP sensitive, being half-blocked by ~10 μmol/l ATP (14). By contrast, estimates of the ATP sensitivity of the channel in intact β-cells suggest a K_i of ~1 mmol/l (15). This is explained by the presence of intracellular agents that reduce ATP sensitivity in the intact cell, but are washed away from the membrane when the patch is excised. Several cytosolic agents are known to modulate ATP sensitivity, including nucleoside diphosphates (ADP and GDP), oleoyl-CoA (16,17), and the membrane phospholipid phosphatidylinositol-4,5-bisphosphate (18–20), with the relative contribution of these factors still remaining to be established.

In addition to ATP, ADP appears to be of preeminent importance in metabolic channel control (21–25). The Mg\(^{2+}\) complex of ADP potently activates the channels by interaction with a nucleotide site residing on SUR1 (24,26), and loss of this effect leads to loss of metabolic regulation (26–29) and the PHHI syndrome (9,27). It has not been proven that adenine nucleotides are the main or sole regulators of the K_ATP channel in intact β-cells, but the variations of channel activity in patches with fluctuation of nucleotide concentrations around the physiological values (30) and evidence that the cytosolic ATP-to-ADP ratio changes over a wide range of glucose concentrations (31,32) support this assumption.

FIG. 1. Stimulation of insulin secretion in the β-cell. A rise in the extracellular glucose concentration increases phosphorylation of glucose by glucokinase (GK), the rate-limiting enzyme for the metabolism of glucose in the β-cell. Glycolysis results in conversion to pyruvate, which preferentially enters the mitochondria and fuels the TCA cycle. The resultant increase in the [ATP/ADP] ratio leads to closure of K_ATP channels, depolarization of the plasma membrane, opening of voltage-gated Ca\(^{2+}\) channels, and an increase of the cytosolic Ca\(^{2+}\) concentration, which triggers the exocytosis of insulin. Sulfonylureas (SUs) inhibit the K_ATP channel and initiate the same chain of events, whereas potassium channel openers (KCOs [e.g., diazoxide]) activate the channel, hyperpolarize the β-cell, lower cytosolic Ca\(^{2+}\), and inhibit insulin secretion. Glucose-6-P, glucose-6-phosphate.

EFFECT OF E23K, A COMMON POLYMORPHISM IN KIR6.2, ON NUCLEOTIDE SENSITIVITY OF PANCREATIC K_ATP CHANNELS

Three common missense single nucleotide polymorphisms (SNPs) have been observed in KIR6.2: E23K, L270V, and I337V (33–37). Their potential impact in type 2 diabetes led us to analyze their functional relevance (38). Whereas L270V and I337V did not have an effect on the properties of reconstituted human SUR1/KATP_6.2 channels, substitution of a lysine (K) for a glutamic acid (E) in position 23 (E23K) markedly affected channel gating by significantly reducing the time spent in long interburst closed states, thus producing a distinct increase in the spontaneous open probability (P_O). Importantly, an in vitro model for the heterozygous genotype (E/K, with E in position 23 of KIR_6.2 in one allele and K in the other) resulted in intermediate P_O values. Consistent with the idea that nucleotide-induced channel inhibition results from interaction with the interburst closed states (39,40), E23K decreased sensitivity toward inhibitory ATP\(^4\) in the models for the heterozygous and homozygous genotypes (K/K, with K in position 23 of KIR_6.2 in both alleles). Stimulation of insulin secretion requires reduction of the P_O of pancreatic β-cell K_ATP channels to values <0.02 (25,41). Both the increased spontaneous P_O values and the reduced ATP sensitivity contributed to a rightward shift of the corresponding ATP concentration (IC_{R2} value) through E23K (38).

These experiments demonstrated that E23K is functionally relevant in significantly modulating spontaneous P_O and ATP sensitivity (38). However, they did not show how channel properties are affected in intact cells, as here additional factors other than the cytosolic concentration of ATP are involved in channel control. Because MgADP appears particularly important among these factors, channel properties were analyzed in the simultaneous presence of activatory and inhibitory nucleotides (30). These experiments indicated that besides reducing sensitivity toward inhibitory ATP, E23K in KIR_6.2 increases the sensitivity of pancreatic β-cell K_ATP channels for activation through nucleoside diphosphates (30).

E23K AND TYPE 2 DIABETES

The role of E23K in type 2 diabetes has been assessed in five population-based studies of Caucasians (33–37). Whereas the earlier studies with modest sample sizes failed to detect the predisposition (33–35), a fourth study (36) that included the results of the first three studies in a meta-analysis showed a clear association of the homozygous state (K in both alleles). This finding was confirmed in a recent analysis (37), yielding an overall P value for the association of 4 × 10\(^{-6}\) (38). The functional significance of E23K was much weaker in an in vitro model for the heterozygous genotype (38), suggesting that in this state, predisposition to type 2 diabetes should be more discrete. Although tending toward an increased risk (33–37), the overall P for association with the heterozygous state consistently remained insignificant (P > 0.05).

Hence, the clear functional relevance and the association of homozygous E23K with type 2 diabetes suggest that by critically affecting the nucleotide sensitivity of K_ATP channels in pancreatic β-cells, this polymorphism induces
a discrete inhibition of insulin secretion, thereby predisposing to the disease (38). This model is consistent with the postulated critical role of an inborn secretory defect in the genesis of type 2 diabetes (42).

The conclusion that E23K predisposes to type 2 diabetes by inducing overactivity of pancreatic \( \beta \)-cell K\(_{\text{ATP}} \) channel activity and thereby inhibiting insulin secretion is strongly supported by a recent study in transgenic mice (43). Those authors demonstrated that discrete targeted reduction of the channels’ ATP sensitivity in pancreatic \( \beta \)-cells is sufficient to induce severe neonatal diabetes in mice. The model is also consistent with a study in healthy young adults indicating that both the homozygous and the heterozygous state were associated with either direct or indirect evidence for reduced glucose-induced insulin secretion (35). However, because KIR6.2 expression is not restricted to pancreatic \( \beta \)-cells (12,13), other effects (e.g., altered glucose sensing in the brain) might still contribute to predisposition.

We then estimated the relative attributable risk of E23K in Caucasians by calculating the odds ratio (OR). In published studies (33–37), ORs for the homozygous genotype ranged from 1.8 to 2.7 (Fig. 2A), yielding a weighted average of 2.11 (\( P < 4 \times 10^{-6} \)). Values for the heterozygous genotype varied from 0.79 to 1.9 (Fig. 2B), with a mean of 1.12 for the combination of all studies (\( P > 0.05 \)). Allelic frequencies of E23K were very similar in the populations screened, ranging from 30 to 38% (Fig. 2C), with a weighted average of 34%. Consistent with Hardy-Weinberg equilibrium, genotypic frequencies averaged at 42 (E/E, homozygous genotype with E in position 23 of KIR6.2 in both alleles), 47 (E/K), and 11% (K/K).

Risk and frequency estimates were used to calculate the proportion of type 2 diabetic patients that would not develop the disease if all subjects had the lower-risk E/E genotype. Taking the calculated average values of 2.11 for the K/K and 1.12 for the E/K genotype, we found that in Caucasians, 10 and 5% of disease cases were attributable to occurrence of these genotypes, respectively. Thus the combination of all published data suggests that in this ethnic group, 15% of type 2 diabetic cases might be attributable to K\(_{\text{ATP}} \) channel overactivity induced by the E23K allele of KIR6.2.

**POTENTIAL ROLE OF E23K IN EVOLUTION**

The high allelic prevalence of E23K (\( f_k \)) in Caucasians with similar values in all populations screened (Fig. 2C) suggests that E23K represents a balanced polymorphism that confers selectionary advantage through fine-tuning of insulin secretion in heterozygotes (38). By reducing glucose uptake in muscle and fat, discrete inhibition of release might result in favorable substrate supply for tissues with insulin-independent uptake; hence, high frequency of the E/K state might have evolved as an adaptation to the human brain. Importantly, this model implies increased susceptibility to type 2 diabetes as the inherent price for the evolutionary benefit of the heterozygous state, and thus E23K provides evidence in support of the “thrifty genotype” hypothesis (44). However, diverging from this...
concept, predisposition might have evolved as a response to altered tissue demands rather than to periodic famine (38).

Recently, we argued that tetrameric $K_{ATP}$ channel stoichiometry should confer protection against diabetic dysregulation resulting from heterozygous mutations in $K_{IR6.2}$ (45). The lower OR for the E/K state supports this idea (Fig. 2A and B). E23K, however, sheds additional light on the impact of channel stoichiometry. While on the one hand this architecture protects against diabetes, on the other hand it imposes a hurdle if evolution seeks to reduce ATP sensitivity. This is due to the dominance of the KIR other hand it imposes a hurdle if evolution seeks to reduce channel stoichiometry. While on the one (Fig. 2

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<th>Wild-type</th>
<th>E23K</th>
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<td>$K_{d6.2}$</td>
<td>0.09 ± 0.04 mmol/l</td>
<td>0.12 ± 0.05 mmol/l</td>
</tr>
<tr>
<td>$I_{max}$</td>
<td>88 ± 2%</td>
<td>86 ± 3%</td>
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<tr>
<td>Hill coefficient</td>
<td>1.28 ± 0.05</td>
<td>1.24 ± 0.04</td>
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Channel inhibition was recorded from inside-out patches, as shown in Fig. 3. Parameters were estimated by fitting the function $P_O = E_{max}/(1 + ((drug)/IC_{50})^p) + (1 - E_{max})$ to the data of each single experiment, where $h$ is the Hill coefficient. Results shown as means ± SE from independent experiments ($n = 5$ each). For further methodical details, see refs. 38 or 55.

**POLYGENIC BASIS OF TYPE 2 DIABETES**

Type 2 diabetes is generally perceived as a polygenic disorder, with disease development being influenced by both hereditary and environmental factors (1,11,42). Genes encoding for key components of insulin secretion and glucose metabolism pathways have been widely considered as targets for defects in type 2 diabetes, and many associations have been reported (49). Besides E23K, three other associations have been confirmed in independent samples: a genetic variation in Calpain 10 (50), a silent SNP in codon 759 of SUR1 (51–53), and a common polymorphism (P12A) in peroxisome proliferator–activated receptor-γ (PPAR-γ) (49,54). Whereas Calpain 10 and the silent SNP in codon 759 of SUR1 were associated with higher risk for type 2 diabetes (50–53), P12A in PPAR-γ was reported to be protective (49,54). Estimates of the weighted attributable proportions in Caucasians are 4, 5, and –6.8%, respectively. Thus, with an estimated attributable proportion of 15%, E23K in $K_{IR6.2}$ appears to be the most important genetic risk factor yet identified.

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