Hyperinsulinism and Diabetes: Genetic Dissection of β Cell Metabolism-Excitation Coupling in Mice

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The role of metabolism-excitation coupling in insulin secretion has long been apparent, but in recent years, in parallel with studies of human hyperinsulinism and diabetes, genetic manipulation of proteins involved in glucose transport, metabolism, and excitability in mice has brought the central importance of this pathway into sharp relief. We focus on these animal studies and how they provide important insights into not only metabolic and electrical regulation of insulin secretion, but also downstream consequences of alterations in this pathway and the etiology and treatment of insulin-secretion diseases in humans.

Introduction: Human Diseases Highlight the Importance of β Cell Metabolism-Excitation Coupling

The pathway from glucose entry into the β cell, through metabolism to electrical signaling and intracellular [Ca2+] (Figure 1), is critical in control of insulin secretion, and it is becoming increasingly apparent that many of the relevant genes underlying human diabetes and hyperinsulinism are found in this pathway. Established steps in response to elevated blood glucose are uptake through glucose transporters, conversion to glucose-6-phosphate by glucokinase, and metabolism to generate ATP. A rise in cytoplasmic [ATP]/[ADP] leads to ATP-sensitive potassium (KATP) channel closure at the plasma membrane, which, in turn, results in membrane depolarization, opening of voltage-dependent Ca2+, and voltage-gated K+ channels (Dukes and Philipson, 1996; Philipson, 1999; Rorsman and Trube, 1986; Su et al., 2001). Calcium entry through Ca2+ channels results in increased intracellular calcium concentration ([Ca2+]i), which, in turn, triggers insulin exocytosis (Ashcroft and Rorsman, 1990; MacDonald et al., 2005).

Congenital hyperinsulinism of infancy (HI), characterized by constitutive insulin secretion despite low blood glucose (Aynsley-Green, 1981), can result from abnormalities in several genes involved in metabolism-excitation (M-E) coupling, including loss-of-function (LOF) mutations in the KATP channel and gain-of-function (GOF) mutations in glucokinase (GCK), glutamate dehydrogenase, or short-chain acyl-CoA dehydrogenase (Palladino et al., 2008). Conversely, permanent and transient neonatal diabetes mellitus (PNDM, TNDM) can result from LOF mutations in glucokinase and GOF mutations in the β cell KATP channel subunits (Smith et al., 2007). Maturity onset diabetes of the young 2 (MODY-2) results from weaker LOF mutations in GCK (Cuesta-Munoz et al., 2004; Gloyn, 2003a), and monogenic diabetes caused by mitochondrial DNA mutation results from defective β cell secretion (Maassen, 2002). Recent genome-wide association studies (GWAS) identified multiple polymorphisms associated with type 2 diabetes (Cauchi et al., 2008; Sladek et al., 2007; Unoki et al., 2008; Yasuda et al., 2008; Zeggini et al., 2007), and again, many are in or near several genes that encode for proteins involved in β cell M-E coupling directly or indirectly via Ca-dependent processes. These include CAPN10 (calpain10, an intracellular calcium-dependent cysteine protease), SLC30A8 (a zinc transporter), KCNJ11 (the pore-forming subunit of the KATP channel), and KCNQ1 (pore-forming subunit of a voltage-gated K channel). Others, including CDKAL1, HHEX, HNF4A, IGF2BP2, PPARG, and TCF7L2, are potentially involved in β cell growth or proliferation and may indirectly affect insulin secretion (Pascoe et al., 2008; Stancakova et al., 2009).

During the last 10 years or so, while these genetic bases of human disease have been identified, detailed knowledge of in vivo β cell function has come from genetically modified mice. We focus on how these studies, in dissecting M-E coupling in vivo, inform islet function in the intact animal and the dysfunctions that result in hyperinsulinism and diabetes. In many cases, different gene products fulfill similar roles in human and mouse β cells, and extrapolation from mouse to man is not automatic. Nevertheless, emerging themes from mouse studies not only provide mechanistic insights to the metabolic regulation of insulin secretion and the downstream consequences of altered genes, but in many cases may directly predict and inform the etiology of the human diseases.

Mouse Models of Altered Glucose Uptake and Glucose Metabolism

Loss of Membrane Glucose Transporters Causes Diabetes

Although both GLUT1 and GLUT2 isoforms may be present in human islets (Richardson et al., 2007), GLUT2 is the main isoform expressed in rodent pancreatic β cells (Efrat et al., 1994; Thorens, 2001). Inactivation of GLUT2 (GLUT2−/−) in mice leads to severe hyperglycemia accompanied by high circulating fatty acids, and GLUT2−/− mice die within the first 3 weeks after birth (Guillam et al., 1997). Isolated islets show loss of first-phase and reduced second-phase glucose-stimulated insulin secretion (GSIS) (Guillam et al., 1997), reflecting a lack of glucose uptake into the β cell. Re-expression of glucose transporters in GLUT2 knockout islets (using recombinant lentiviral expression or by crossing with mice overexpressing GLUT2 or GLUT1 under Rip...
of intracellular [Ca2+], which stimulates insulin secretion. Voltage-dependent KATP channels (Page et al., 1992; Sakura et al., 1998). Reiterating key features, mice overexpressing yeast hexokinase (Epstein et al., 1992a) or the GCK gene itself (Shiota et al., 2001) are relatively hyperinsulinemic. Conversely, homozygous inactivating GCK mutations cause PNDM (Gloyn et al., 2002; Gloyn, 2003a; Njolstad et al., 2001). Mice with deletion of the GCK gene in pancreatic β cells and neurons, but not in the liver (by targeted disruption of the appropriate exon 1 variant), showed severe perinatal diabetes and died shortly after birth (Grupe et al., 1995; Terauchi et al., 1995). Confirming the critical role of β cell GCK, transgenic re-expression only in β cells reversed diabetes in ~50% of the mice (Grupe et al., 1995).

Whereas homozygous LOF GCK mutations cause PNDM (Gloyn, 2003a), heterozygous LOF mutations, resulting in only partial reduction of GCK activity, underlie MODY-2 (Cuesta-Munoz et al., 2004; Gloyn, 2003a), characterized by impaired insulin secretion and diabetes onset in early adulthood, typically not worsening over time (Fajans et al., 2001). Heterozygous β cell GCK+/− mutant mice show glucose intolerance and impaired GSIS (Bali et al., 1995; Grupe et al., 1995; Sakura et al., 1998), but again, the diabetes does not progress, reiterating the MODY-2 phenotype. Why the phenotype is nonprogressive in humans and mice is interesting, given that the level of hyperglycemia that is reached (typically fasted blood glucose of ~125–135 mg/dl; Codner et al., 2006; Fajans et al., 2001; Shehadeh et al., 2005) is one that progresses to uncontrollable levels in other forms of diabetes, including human type 2 (Guillausseau et al., 2008; Matveyenko and Butler, 2008). Insulin levels are typically normal, such that decreased insulin/glucose ratio suggests increased insulin sensitivity in humans and mice (Grupe et al., 1995; Katagiri et al., 1992; Terauchi et al., 1995).

Chronic (48–96 hr) exposure of wild-type islets induces dysfunction (Sreenan et al., 1998). As we discuss below, an emerging idea from cessation of insulin secretion. Factors that impair ATP production or downstream signaling are expected to suppress GSIS. Several genes that directly or indirectly alter ATP production and therefore underlie a diabetic or hyperinsulinemic phenotype when mutated (humans and/or mouse models) are shown in color: glucose transporter 2 (GLUT2), glucokinase (GK), nicotinamide nucleotide transhydrogenase (Nnt), uncoupling protein 2 (UCP2), mitochondrial DNA mutations (mtDNA), and glutamate dehydrogenase (GDH). Mutations that may indirectly affect channel activity are also shown.

**Figure 1. Schematic Illustration of the Glucose-Stimulated Insulin Secretory Pathway in β Cells**

(A) Hematoxylin and eosin-stained paraffin section of mouse pancreas. The pancreas is composed of exocrine tissue and endocrine tissue (islet of Langherans). Islets contain different cell types, including the insulin-secreting β cells. Arrows point to exocrine and endocrine tissue. (B) Schematic illustration of the β cell glucose-stimulated insulin secretion pathway. Glucose entering the β cell through glucose transporters (GLUT2) is phosphorylated by glucokinase (GK) and metabolized by glycolysis (cytoplasm) and tricarboxylic acid (TCA) cycle (mitochondria). A rise in the [ATP]/[ADP] ratio resulting from oxidative metabolism inhibits the ATP-sensitive K+ channels (KATP) at the cell surface, causing membrane depolarization and opening of voltage-dependent Ca2+ channels (VDCC). This results in a rise of intracellular [Ca2+], which stimulates insulin secretion. Voltage-dependent outward K+ channels (Kv) are involved in membrane repolarization and control) restores normal GSIS (Guillam et al., 2000; Thorens et al., 2000).

**Alterations in Glucokinase Activity Cause Diabetes and Hyperinsulinism**

Following entry into the β cell, glucose is phosphorylated by GCK and then metabolized through glycolytic and oxidative pathways (Matschinsky, 1996) (Figure 1B). In humans, GOF mutations in either GCK or mitochondrial glutamate dehydrogenase (GDH) cause HI, elevating [ATP]/[ADP] at any ambient glucose concentration and suppressing KATP channel activity (Page et al., 1992; Sakura et al., 1998). Reiterating key features, mice overexpressing yeast hexokinase (Epstein et al., 1992a) or the GCK gene itself (Shiota et al., 2001) are relatively hyperinsulinemic. Conversely, homozygous inactivating GCK mutations cause PNDM (Gloyn et al., 2002; Gloyn, 2003a; Njolstad et al., 2001). Mice with deletion of the GCK gene in pancreatic β cells and neurons, but not in the liver (by targeted disruption of the appropriate exon 1 variant), showed severe perinatal diabetes and died shortly after birth (Grupe et al., 1995; Terauchi et al., 1995). Confirming the critical role of β cell GCK, transgenic re-expression only in β cells reversed diabetes in ~50% of the mice (Grupe et al., 1995).

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multiple transgenic animals is that secondary progression of diseases may depend critically on the underlying cellular mechanisms. “Underexcited” islets, such as those with reduced metabolic flux, may respond well to chronic restoration of excitability, whereas “hyperstimulation” of otherwise normal islets may be detrimental.

A first polygenic mouse model of noninsulin-dependent diabetes mellitus (Terauchi et al., 1997) was obtained by crossing heterozygous GCK+/− mice (Terauchi et al., 1995) with homozygous insulin receptor substrate 1 knockout (IRS-1−/−) mice. Parental IRS-1−/− mice were insulin resistant but did not develop diabetes due to compensatory β cell hyperplasia (Araki et al., 1994; Tamemoto et al., 1994). Importantly, GCK+/−/IRS-1−/− mice showed hyperglycemia despite β cell hyperplasia and became overtly diabetic with age (Terauchi et al., 1997), experimentally confirming that genetic combination of insulin resistance and reduced insulin secretion can lead to diabetes.

Any consequences of GCK deficiency on electrical activity should be absent in animals that lack glucose-regulated KaTPr channels. Perinatal lethality was avoided in GCK−/−/Kir6.2−/− double knockout mice, although they were still insulin deficient and still died prematurely (Remedi et al., 2005). However, heterozygous GCK+/− animals, which are markedly diabetic on the Kir6.2 wild-type background, became only mildly glucose intolerant on the Kir6.2−/− background, essentially behaving like Kir6.2−/− mice (see below) (Remedi et al., 2005), confirming that a major feature of GCK deficiency is indeed reduced excitability via enhanced KaTPr channel activity.

**Alteration of Mitochondrial Proteins Involved in Regulation of ATP Production and Expenditure: Indirect Action via Electrical Signaling**

Mitochondria provide the major ATP-generating capacity of β cells (Erecinska et al., 1992; Maechler and Wollheim, 2001). Overactive mitochondrial metabolism will increase cytosolic [ATP]:[ADP], leading to closure of β cell KaTPr channels and enhancing secretion (see below) (Figure 1). Conversely, impaired mitochondrial metabolism will decrease [ATP]:[ADP], preventing KaTPr channel closure and suppressing secretion (Wallace, 2001). Uncoupling proteins (UCPs) dissociate mitochondrial substrate oxidation from ATP synthesis (Figure 1). UCP2 activation contributes to decreased reactive oxygen species (ROS) formation and free fatty acid transport/metabolism but mostly reduces the efficiency of ATP generation (Arsenijevic et al., 2000; Himms-Hagen and Harper, 2001; Nishikawa et al., 2000; Samec et al., 1998). UCP2 is present in β cells, and consistent with above predictions, UCP2−/− mice demonstrate increased circulating insulin, without changes in peripheral insulin sensitivity (Joseph et al., 2002; Zhang et al., 2001). UCP2−/− islets show increased [ATP] and hypersecretion of insulin at any glucose concentration (Joseph et al., 2002; Zhang et al., 2001). When fed a high-fat diet, these mice show improved β cell glucose sensitivity, enhanced GSIS, and increased insulin content (Joseph et al., 2002). Several animal models support the idea that UCP2 upregulation and consequent inefficient ATP production is a feature of glucolipotoxic conditions (Chan et al., 1999). Mice and rats fed a high-fat diet show upregulation of UCP2 gene expression and elevated plasma lipids (Briand et al., 2002; Chan et al., 2001). Fa/fa rats (leptin-receptor deficient) and ob/ob mice (leptin deficient) are popular models of obesity-induced diabetes, and both show upregulation of UCP2 mRNA in β cells (Kassis et al., 2000; Zhang et al., 2001). Knockout of the UCP2 gene in the ob/ob mouse background improved GSIS and reduced blood glucose (Zhang et al., 2001).

In C57Bl6 mice, reported by some groups to exhibit impaired insulin secretion relative to other strains (Toye et al., 2005), mutations in the nicotinamide nucleotide transhydrogenase (Nnt) have been identified. Nnt is a nuclear-encoded mitochondrial protein catalyzing reversible reduction of NADP+ by NADH and promoting proton translocation across the membrane and detoxification of ROS (Arkblad et al., 2005; Hoek and Rydstrom, 1988). Accumulation of ROS in the mitochondrial matrix can increase UCP2 activity, enhancing proton leakage and impairing ATP production (Echtay et al., 2002; Krauss et al., 2003). Nnt-transgenic mice showed impaired glucose tolerance and loss of both β cell ATP synthesis and GSIS, consistent with a direct effect of impaired ATP production on KaTPr channel activity (Freeman et al., 2006). No mutations in mitochondrial enzymes have been reported in human diabetes, but a few mutations in mitochondrial DNA (mtDNA) have. Mitochondria are a major source of free radicals, as a result of electrons leaking into the mitochondrial matrix reacting with molecular oxygen. An A3243G mutation in the tRNA(Leu(UUR)) gene was identified as a common diabetes-causing mitochondrial DNA mutation. Mutation carriers exhibit reduced first- and second-phase insulin secretion (Maassen et al., 2004), although the underlying molecular mechanisms are unclear. A mouse model of mitochondrial diabetes was generated by disruption of the nuclear gene Tfam, a transcriptional activator essential for mtDNA expression in β cells (Silva et al., 2000). These mice showed depletion of mtDNA and developed diabetes at 5 weeks. Again, consistent with impaired M-E coupling, dispersed β cells demonstrated hyperpolarized mitochondria, impaired Ca2+ signaling, and decreased GSIS. As in other models in which the primary defect is in M-E coupling, older Tfam mutant mice showed reduced β cell mass, indicating secondary progression (Silva et al., 2000). Recently, a transgenic mouse expressing mutant mtDNA polymerase γ (D181A polyγ) under insulin promoter control was generated (Bensch et al., 2009) to cause accumulation of mtDNA mutations in β cells. Despite males being overtly diabetic and females being glucose intolerant by 6 weeks of age, GSIS was normal in isolated islets, suggesting a non-islet mechanism.

Oxygen-sensing pathways have also been implicated in modulation of insulin secretion: both β cell-specific (βHvKO) and whole-pancreas (VhlKO) knockout of von Hippel-Lindau (VHL) protein, which controls the degradation of hypoxia-inducible factor (HIF), are glucose intolerant. Isolated islets show altered expression of β cell glucose transporter and glycolytic genes, impaired glucose uptake and metabolism, and reduced GSIS (Cantley et al., 2009; Zehetner et al., 2008). Hif1α is expressed in these β cells, and deletion of Hif1α restores GSIS in these mice (Cantley et al., 2009; Zehetner et al., 2008). β-cell-specific Hif-1α deficient mice hypersecrete insulin at low glucose concentration but have impaired GSIS and increased [Ca2+] (Cantley et al., 2009; Zehetner et al., 2008). Thus, impaired mitochondrial oxidation and/or oxygen-sensing mechanisms may all contribute to altered secretion via altered M-E coupling.
Genetic Manipulation of $K_{\text{ATP}}$ Channels: Mouse Models of Hyperinsulinism and Diabetes

$K_{\text{ATP}}$ Knockout and Dominant-Negative Mice as Models of Hyperinsulinemia

$\beta$ cell $K_{\text{ATP}}$ channels are complexes of the sulfonylurea receptor 1 (SUR1, ABCC8) and the potassium channel Kir6.2 (KCNJ11) (Clement et al., 1997; Inagaki et al., 1995; Sakura et al., 1995; Shyng and Nichols, 1997). In humans, LOF mutations in these genes are major causes of HI (Aguilar-Bryan et al., 2001; Dunne et al., 2004; Huopio et al., 2002a; Stanley, 2002). Reduction of channel expression at the cell surface, loss of MgADP stimulation, or abolished channel activity are primary defects (Dunne et al., 1997; Nestorowicz et al., 1998; Nichols et al., 1996; Shyng et al., 1998). Kir6.2$^{-/-}$ and SUR1$^{-/-}$ mice (Miki et al., 1998; Seghers et al., 2000; Shiota et al., 2002), as well as mice expressing a dominant-negative Kir6.2 mutant transgene in $\beta$ cells (Kir6.2[AAA] [Koster et al., 2002] or Kir6.2[G132S] [Miki et al., 1997], both of which disrupt the selectivity filter) have been generated. Kir6.2$^{+/-}$ and SUR1$^{+/-}$ mice (Miki et al., 1998; Seghers et al., 2000; Shiota et al., 2002) lacking $K_{\text{ATP}}$ in multiple tissues and Kir6.2[G132S] mice that specifically lack $\beta$ cell $K_{\text{ATP}}$ (Miki et al., 1997) show a complex and surprising phenotype that does not trivially replicate human HI. These mice reportedly demonstrate transient hyperinsulinemia and hypoglycemia as neonates, but islets from adult SUR1$^{-/-}$ or Kir6.2$^{-/-}$ mice show a dramatic loss of insulin secretion at all glucose concentrations, and the animals are relatively hypoinsulinemic (Miki et al., 1998; Seghers et al., 2000; Shiota et al., 2002). In Kir6.2[G132S] mice, loss of $\beta$ cell mass was reported (Miki et al., 1997), although hyperglycemia was apparently spontaneously improved and insulin content even increased in older mice (Oyama et al., 2006).

However, $\beta$-cell-specific Kir6.2[AAA] dominant-negative mice, which lose $K_{\text{ATP}}$ channel activity in only $\sim$70% of $\beta$ cells, exhibit elevated circulating insulin levels that persist through adulthood, with essentially normal insulin content and islet morphology (Koselev, 2004; Yang and Berggren, 2005a, 2005b). Cav1.2 and Cav1.3 may be the dominant subunits (Barg et al., 2001; Burger et al., 2001), additional $K_{\text{ATP}}$ GOF (Kir6.2[N2-30]) transgenic mouse lines with lower levels of transgene expression exhibit normal islet morphology and insulin content but are glucose intolerant (and more so on high-fat diet) (Koster et al., 2006), reiterating the general phenotype of humans carrying the E23K polymorphism (Villareal et al., 2009).

Control-based genetic studies (Gloyn, 2003b), metanalysis (Nielsen et al., 2003; Schwanstecher et al., 2002), and GWAS (Saxena et al., 2007; Zeggini et al., 2007) have established the E23K polymorphism in Kir6.2 as a risk factor in type 2 diabetes. Although early studies failed to demonstrate a significant effect of E23K on recombinant $K_{\text{ATP}}$ channel properties (Riedel et al., 2003; Sakura et al., 1996), subsequent studies reveal a small rightward shift in ATP sensitivity (Schwanstecher et al., 2002; Villareal et al., 2009). Such GOF behavior is predicted to suppress GSIS, consistent with the findings in nondiabetic individuals who carry this polymorphism (Chistjakov et al., 2009; Flores et al., 2004; Nielsen et al., 2003; Villareal et al., 2009).

Mouse Models of Altered Ca Channels

The Role of L-Type High-Voltage-Activated Ca Channels in Hyperinsulinemia and Diabetes

The Ca$_{\text{a}}$1 subfamily, comprising four members (Ca$_{\text{a}}$1.1 to Ca$_{\text{a}}$1.4) (Hofmann et al., 1999), generates L-type voltage-dependent Ca channels (VDCCs) involved in insulin secretion (Ichi et al., 2005; Mears, 2004; Yang and Berggren, 2005a, 2005b). Ca$_{\text{a}}$1.2 and Ca$_{\text{a}}$1.3 may be the dominant subunits (Barg et al., 2001; Iwashima et al., 1993; Yang et al., 1999) (Figure 1). Ca$_{\text{a}}$1.3$^{-/-}$ mice are viable with no major disturbances in insulin secretion even though they show deafness, bradycardia, and arrhythmias (Platzer et al., 2000), suggesting that Ca$_{\text{a}}$1.3 is not normally a major player in mouse $\beta$ cell insulin secretion. Ca$_{\text{a}}$1.2$^{-/-}$ mice die in utero before day 15, presumably because of lack of functional VDCC in the heart (Seisenberger et al., 2000), obviating
study of β cell roles, but β-cell-specific knockout (β-CaV1.2+/−) mice (deleted exons in one allele of CaV1.2 and “floxed” exons in the other, using Cre/loxP recombination) exhibit decreased VDCC and a marked reduction in insulin exocytosis in response to initial membrane depolarization (first-phase) but unaffected second-phase secretion (Schulla et al., 2003). The β cell CaV1.2 is sensitive to dihydropyridines (DHPs), and these drugs almost abolish GSIS in β cell lines (Minami et al., 2002). CaV1.2+/− β cells show no DHP-sensitive VDCC, but glucose-induced [Ca2+]i oscillations and action potential (AP) firing persist, suggesting that L-type channels are necessary for insulin secretion, but not for electrical activity and [Ca2+]i oscillations, perhaps indicating compensatory overexpression of non-L-type channels in β cells lacking Cav1.2 (Schulla et al., 2003). Timothy syndrome, due to CaV1.2 overactivity, is characterized by deafness, webbing of fingers and toes, congenital heart disease, and severe arthropathies and is associated with hypoglycemia (Szlafszki et al., 2004), probably due to CaV1.2 overactivity in β cells. It seems likely that LOF polymorphisms or mutations of L-type VDCC in humans should be associated with diabetic syndromes, but none have yet been identified, and we speculate that this is again because of compensatory upregulation of other subunits.

**Non-L-Type High-Voltage-Activated Ca Channels in the Regulation of Insulin Secretion**

The role of non-L-type, high-voltage-activated Ca channels (e.g., Cav2.3 R-type Ca channel) in β cells is controversial, and some studies indicate their absence in human islets (Braun et al., 2008; Yang and Berggren, 2005b). But there is evidence for association of CaV2.3 polymorphisms with impaired insulin secretion and development of type 2 diabetes (Holmkvist et al., 2007). They appear to play a role in second-phase insulin secretion by mobilizing insulin reserve granules to the readily releasable pool in mice. CaV2.3 (CACNA1E) knockout (CaV2.3−−/−) mice show impaired glucose tolerance and GSIS (Perevezev et al., 2002). In addition, genetic or pharmacological (using R-type channel blocker SNX482) ablation of CaV2.3 channels strongly suppresses second-phase secretion, but the first phase is unaltered (Jing et al., 2005). Pancreatic cells from CaV2.3−−/− mice show reduction of Ca2+ signaling and impairment of insulin granule recruitment after the initial exocytotic burst (Jing et al., 2005).

**Voltage-Dependent Outward K+ Current: Repolarization of β Cells and Insulin Secretion**

Pancreatic β cells have prominent voltage-dependent outward K+ (Kv) currents that mediate AP repolarization (Dukes and Philipson, 1996; Philipson, 1999; Rosman and Trube, 1986; Su et al., 2001), and both Kv1 (KCNA) and Kv2 (KCNB) channel families are involved (MacDonald et al., 2001) (Figure 1C). Kv2.1 expression is high in β cells (MacDonald et al., 2001; Roe et al., 1996), and adenovirus-mediated expression of dominant-negative Kv2.1 reduced (60%–70%) β cell delayed rectifier currents and markedly enhanced GSIS (MacDonald et al., 2001). Perfusion of mouse pancreas with Kv2.1 antagonists enhances first- and second-phase insulin secretion but, as expected, does not affect basal insulin levels (MacDonald et al., 2002). Philipson and colleagues (Philipson et al., 1994) generated Kv2.1-overexpressing transgenic mice under Rip control. Although expression levels were variable, the mice showed hyperglycemia and hypoinsulinemia, with normal β cell morphology and insulin content (Philipson et al., 1994). Conversely, Kv2.1 knockout (Kv2.1−−/−) mice show reduced fasting blood glucose level and elevated serum insulin (Jacobson et al., 2007), with enhanced GSIS. Isolated Kv2.1−−/− β cells exhibit increased glucose-induced AP duration, but firing frequency is diminished (Jacobson et al., 2007), consistent with loss of Kv currents and enhanced Ca2+ entry during the AP, leading to increased insulin secretion. SNARE proteins also affect insulin exocytosis (Daniel et al., 1999). Syntaxin-1A (STX-1A) directly binds and regulates Ca2+ channels (Yang et al., 1999), KATP (Kang et al., 2002), and Kv2.1 channels in β cells (Leung et al., 2003, 2005). Transgenic mice overexpressing STX-1A in β cells demonstrate fasting hyperglycemia and reduced plasma insulin (Lam et al., 2005), with reduced Ca2+ currents but little change in the Kv and KATP currents (Lam et al., 2005).

Multiple other K channels may be involved in insulin secretion and may ultimately be shown to be involved in secretion defects. Variants in the voltage-gated potassium channel KCNQ1 gene have recently been associated with susceptibility to type 2 diabetes, impaired fasting glucose, and β cell function in various populations (Hu et al., 2009; Tan et al., 2009; Unoki et al., 2008; Yasuda et al., 2008). KCNQ1−−/− mice demonstrate enhanced insulin sensitivity and glucose tolerance, as well as decreased fed and fasting glucose and insulin levels (Boini et al., 2009), broadly consistent with a role in secretion, but the evidence for KCNQ1 expression in islets is weak (Jonsson et al., 2009; Mussig et al., 2009; Stancakova et al., 2009), and further investigation is needed. The evidence that the KCNQ1 locus harbors control elements that influence the imprinting of neighboring genes (Lee et al., 1997; Smillinich et al., 1999), some associated with type 2 diabetes, suggests that the mechanism responsible for type 2 diabetes susceptibility may extend beyond the direct effect of KCNQ1 (McCarthy, 2008). The role of Ca-activated potassium channels in β cell function remains controversial. Early studies demonstrated the presence of Ca2+-dependent K+ conductances in β cells (Atwater et al., 1983), and recent studies have argued for roles of small conductance SK channels in rodent β cells (Tamarina et al., 2003); large conductance BK channels may also be prominent in human β cells (Braun et al., 2008), but again, more studies are clearly needed.

**Genetic Alterations of Calmodulin in Hyperglycemia and Neonatal Diabetes**

Calmodulin and protein kinase C are two β cell calcium-binding proteins with multiple potential roles in secretion (Hubinont et al., 1984; Pretniki and Matschinsky, 1987; Sugden et al., 1979). Calmodulin may be a major calcium buffer, and transgenic mice that overexpress calmodulin (CaM) specifically in pancreatic β cells develop severe diabetes within hours after birth, demonstrating elevated blood glucose and glucagon levels and a marked reduction in insulin levels (Epstein et al., 1992b), very much like that seen in GOF Kir6.2 transgenic mice (Remedi et al., 2009). A similar phenotype was found in transgenic mice overexpressing inactive CaM-8 and containing a deletion of eight amino acids in the central helix (Ribar et al., 1995), suggesting a mechanism involving Ca2+ buffering rather than downstream calmodulin signaling and consistent with Ca2+-sensitive pools of insulin granules: vesicles colocalized with VDCC (Pertusa et al., 1999).
Integrating the Models: Emerging Themes and Future Directions

M-E Coupling Leads to Ca\(^{2+}\): So What?

In a 2002 review, we systematically compared the phenotypic consequences of transgenic manipulation of multiple genes in pancreatic \(\beta\) cells (Nichols and Koster, 2002). Although genes obviously involved in pancreatic development lead to loss of \(\beta\) cells and inevitably insulin-dependent diabetes, many signaling molecules have relatively mild consequences, except for those involved directly in coupling glucose uptake, through metabolism, to electrical signaling (e.g., GLUT2, GCK, Kir6.2, SUR1). In these cases, even minor over- or underactivity leads to under- or oversecretion and marked hyperinsulinism or diabetes. This is dramatic in the case of \(K_{\text{ATP}}\), in which even relatively small shifts in the ATP sensitivity lead to severe diabetes (Girard et al., 2009; Koster et al., 2000; Remedi et al., 2009), glucokinase knockout (Groupe et al., 1995; Terauchi et al., 1995), or calmodulin overexpression (Epstein et al., 1992b; Ribar et al., 1995). The data from human NDM patients are entirely consistent: monogenic NDM is caused by loss of glucokinase et al., 1995). The data from human NDM patients are entirely consistent: monogenic NDM is caused by loss of glucokinase activity, through excitability to elevated \([\text{Ca}^{2+}]_i\) is critical and the pathway from glucose metabolism, initiated by glucokinase remain refractory to stimulation and intracellular \([\text{Ca}^{2+}]\) remains maintained elevated \([\text{Ca}^{2+}]_i\). Although this might be ascribed from these animals will, therefore, provide models for future studies of the mechanisms of glucotoxic loss of \(\beta\) cells and insulin content in the face of low intracellular \([\text{Ca}^{2+}]\). M-E Coupling Leads to Ca\(^{2+}\): Surprises, Too

A clear picture emerging from animal models is that reduced M-E coupling leads to reduced \([\text{Ca}^{2+}]_i\), and reduced insulin secretion, with remarkably small tolerance limits, and this is borne out in humans by the finding that NDM results from relatively small changes in ATP sensitivity of the \(K_{\text{ATP}}\) channel. But there are surprises on the other side of the “see-saw.” The lack of hyperinsulinism (in fact, relative hypoinsulinism) in mice completely lacking \(K_{\text{ATP}}\) has the expected effect of shifting secretion to lower glucose levels, leading to enhanced glucose tolerance, but below a certain level (<~15% of normal \(K_{\text{ATP}}\)) (Nichols et al., 2007; Remedi et al., 2006), there is crossover to marked underscission due to downregulation of secretion itself in the face of a maintained elevated \([\text{Ca}^{2+}]_i\). Although this might be ascribed to some form of \(\text{Ca}^{2+}\) toxicity, it appears to be very labile and readily reversible; the phenotype is closely replicated by chronic pharmacological block of channel activity in mice implanted with slow-release high-dose glibenclamide pellets. Yet this phenotype is completely reversed within hours of removal of the drug (Remedi and Nichols, 2008). Again, such findings point
to previously unconsidered mechanisms and may be of relevance to humans. First, as discussed, there are reports that HI patients with LOF KATP mutations can cross over to a glucose-intolerant phenotype later in life, even without pancreatectomy (de Lonlay et al., 2002; Dunne et al., 2004; Mazor-Aronovitch et al., 2009). Second, chronic sulfonylurea treatment inevitably fails in type 2 patients, again potentially involving chronic down-regulation of secretion that can be reversed by β cell “rest” (Guldstrand et al., 2002; Pfutzner et al., 2006; Sargsyan et al., 2008).

Concluding Remarks
Clearly, mice and humans are not genetically the same, and differential islet morphology is a relevant issue. We do not suggest that animal models can supplant clinical experience or that the implications from animal models can automatically translate to clinical relevance. However, many major players in M-E coupling are likely to be the same in humans and mouse, and recent studies show marked parallels in disease etiology and progression resulting from their derangement in mouse and man. Given the undisputed experimental advantages of the mouse, both genetically and physiologically, mouse models will continue to provide important avenues for explaining basic mechanisms of M-E coupling, but also the molecular basis of and therapeutically possible treatments for treating diseases that result from M-E coupling derangement.

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