Diverse Roles of K_{ATP} Channels Learned From Kir6.2 Genetically Engineered Mice

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The regulation of insulin secretion from pancreatic β-cells depends critically on the activities of their plasma membrane ion channels. ATP-sensitive K^+ channels (K_{ATP} channels) are present in many cells and regulate a variety of cellular functions by coupling cell metabolism with membrane potential. The activity of the K_{ATP} channels in pancreatic β-cells is regulated by changes in the ATP and ADP concentrations (ATP/ADP ratio) caused by glucose metabolism. Thus, the K_{ATP} channels are the ATP and ADP sensors in the regulation of glucose-induced insulin secretion. K_{ATP} channels are also the target of sulfonylureas, which are widely used in the treatment of type 2 diabetes. Molecular cloning of the two subunits of the pancreatic β-cell K_{ATP} channel, Kir6.2 (an inward rectifier K^+ channel member) and SUR1 (a receptor for sulfonylureas), has provided great insight into its structure and function. Kir6.2 subunits form the K^+ ion-permeable pore and primarily confer inhibition of the channels by ATP, while SUR1 subunits confer activation of the channels by MgADP and K^+ channel openers, such as diazoxide, as well as inhibition by sulfonylureas. The SUR1 subunits also enhance the sensitivity of the channels to ATP. To determine the physiological roles of K_{ATP} channels directly, we have generated two kinds of genetically engineered mice: mice expressing a dominant-negative form of Kir6.2 specifically in the pancreatic β-cells (Kir6.2G132S Tg mice) and mice lacking Kir6.2 (Kir6.2 knockout mice). Studies of these mice elucidated various roles of the K_{ATP} channels in endocrine pancreatic function: *1*) the K_{ATP} channels are the major determinant of the resting membrane potential of pancreatic β-cells, *2*) both glucose- and sulfonylurea-induced membrane depolarization of β-cells require closure of the K_{ATP} channels, *3*) both glucose- and sulfonylurea-induced rises in intracellular calcium concentration in β-cells require closure of the K_{ATP} channels, *4*) both glucose- and sulfonylurea-induced insulin secretions are mediated principally by the K_{ATP} channel–dependent pathway, *5*) the K_{ATP} channels are important for β-cell survival and architecture of the islets, *6*) the K_{ATP} channels are important in the differentiation of islet cells, and *7*) the K_{ATP} channels in glucose-responsive cells generally participate in coupling glucose sensing with cell excitability. Interestingly, despite the severe defect in glucose-induced insulin secretion, Kir6.2 knockout mice show only a very mild impairment in glucose tolerance. However, when the knockout mice become obese with age, they develop fasting hyperglycemia and glucose intolerance, while neither fasting hyperglycemia nor glucose intolerance is evident in the aged knockout mice without obesity, suggesting that both the genetic defect in glucose-induced insulin secretion and the acquired insulin resistance due to environmental factors are necessary to develop diabetes in Kir6.2 knockout mice. Thus, Kir6.2G132S Tg mice and Kir6.2 knockout mice provide a model of type 2 diabetes and clarify the various roles of K_{ATP} channels in endocrine pancreatic function. *Diabetes* 49:311–318, 2000

Pancreatic β-cells play a central role in maintaining blood glucose levels within a narrow range by regulating insulin secretion in response to nutrients, hormones, and neurotransmitters. The regulation of glucose-induced insulin secretion depends critically on glucose metabolism and the electrical activity controlled by the various plasma membrane ion channels in pancreatic β-cells (1). Among these, K_{ATP} channels are critical, because they link glucose metabolism to electrical activity of the cells (2–4).

K_{ATP} channels, discovered originally in cardiac myocytes (5), were later found in a variety of tissues, including pancreatic β-cells, pituitary, skeletal and smooth muscles, brain, and kidney (6). K_{ATP} channels couple cell metabolism with membrane electrical excitability (6). Metabolic changes in the cells induce changes in the concentrations of ATP and MgADP, which inhibit and activate K_{ATP} channels, respectively. Thus, K_{ATP} channels are thought to function as ATP and ADP sensors in the regulation of cellular functions.

Classical plasma membrane K_{ATP} channels are now known to consist of two structurally unrelated subunits: an inward rectifier α-subunit, Kir6.2, and a sulfonylurea receptor β-subunit, SUR1 or SUR2 variant, that belongs to the ATP-binding cassette transporter superfamily (7–9). The pancreatic β-cell K_{ATP} channels comprise Kir6.2 and SUR1, while cardiac and skeletal muscle K_{ATP} channels comprise Kir6.2 and SUR2A.

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$[Ca^{2+}]_i$, intracellular calcium concentration; GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide 1; IP_3, 1,4,5-triphosphate; PHHI, persistent hyperinsulinemic hypoglycemia of infancy; PKA, protein kinase A; PP, pancreatic polypeptide; VDCC, voltage-dependent calcium channel; VMH, ventromedial hypothalamus.

Although many important roles of K_{ATP} channels in cell function have been proposed (3,10–12), direct evidence has not been provided. We have generated two kinds of Kir6.2 genetically engineered mice: mice expressing a dominant-negative form of Kir6.2 specifically in pancreatic β-cells (Kir6.2G132S Tg mice) (13) and mice lacking Kir6.2 (Kir6.2 knockout mice) (14). By the study of these mice, many physiologically important roles of K_{ATP} channels have been clarified. Kir6.2 knockout mice also provide a model of the development of type 2 diabetes. We shall discuss the diverse roles of K_{ATP} channels in the endocrine pancreas learned from these genetically engineered mice.

GENERAL FEATURES OF KIR6.2

Kir6.2 cDNA was isolated originally by homology cloning using Kir6.1 cDNA (15) and belongs to the Kir6.0 subfamily of the inward rectifier (Kir) family (16). Human Kir6.2 is a protein of 390 amino acids and shares 71% amino acid identity with human Kir6.1. Kir6.2 has a feature characteristic of the inward rectifier K^+ channel member's two membrane spanning segments (M1 and M2). A region called H5 (or P) between the segments is responsible for K^+ ion selectivity. Like Kir6.1, Kir6.2 has the Gly-Phe-Gly motif in the H5 region. Kir6.2 is expressed at high levels in pancreatic islets, MIN6 cells, and HIT-T15 cells, and at low levels in heart, skeletal muscle, brain, and RINm5F by Northern blot analysis. Kir6.2 and SUR1 are coexpressed in all types of cells in pancreatic islets (17,18). In situ hybridization shows Kir6.2 mRNA to be widely distributed throughout mouse brain (19,20), and it is highly expressed in the paraventricular hypothalamus and ventromedial hypothalamus (VMH) (20). Kir6.2 is also present in glucose-responsive enteric neurons (21). The β-cell K_{ATP} channel is a hetero-octamer consisting of four Kir6.2 subunits and four SUR1 subunits (22–24) (Fig. 1). Both the Kir6.2 and the SUR1 subunits are normally required for functional expression of the K_{ATP} channels; however, removal of 26 or 36 amino acids from the COOH-terminus of Kir6.2 (Kir6.2ΔC26 or Kir6.2ΔC36) produces K_{ATP} channel currents in the absence of SUR1, although such truncated proteins are less sensitive to ATP than the wild-type Kir6.2/SUR1 channel (25). This puzzle has been solved recently by a study showing that the amino acid motif Arg-Lys-Arg located in the COOH-terminal region of Kir6.2 serves as an endoplasmic reticulum retention signal that prevents trafficking to the plasma membrane (26). This signal is masked during formation of the K_{ATP} assembly with Kir6.2 and SUR1. Removal of the Arg-Lys-Arg sequence by deletion of the COOH-terminus of Kir6.2 (Kir6.2ΔC26 or Kir6.2ΔC36) allows surface expression of the truncated Kir6.2 to generate ATP-sensitive K^+ currents without SUR1. The COOH-terminal region of SUR1 has also been shown to be required for the trafficking of the K_{ATP} channels to the plasma membrane. In addition to forming the K^+ ion-permeable pore, Kir6.2 subunits primarily confer inhibition of the channels by ATP (25). On the other hand, SUR1 subunits confer activation of the channels by MgADP and K^+ channel openers such as diazoxide, as well as inhibition by sulfonylureas, and also enhance the sensitivity of the channels to ATP (27–29).

Kir6.2 can be phosphorylated at Ser residue 372 by protein kinase A (PKA), and the phosphorylation of Kir6.2 increases the activity of β-cell K_{ATP} channels (30). On the other hand, SUR1 can be phosphorylated at Ser residue 1571, which is present only in the human species, and the phosphorylation of SUR1 affects channel kinetics, including burst duration and interburst interval and surface expression of the channels (30). However, the physiological significance of PKA-mediated phosphorylation is not clear.

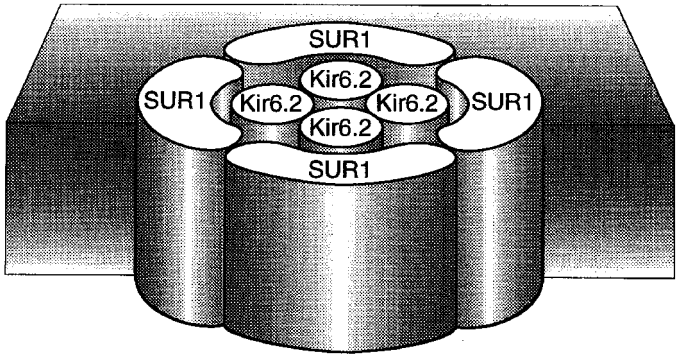

FIG. 1. Subunit assembly of pancreatic β-cell K_{ATP} channel. The β-cell K_{ATP} channel is a hetero-octamer of Kir6.2 and SUR1 with 4:4 stoichiometry.

MUTATIONS OF KIR6.2 IN FAMILIAL PERSISTENT HYPERINSULINEMIC HYPOGLYCEMIA OF INFANCY

Familial persistent hyperinsulinemic hypoglycemia of infancy (PHHI) (ref. #256450 in Online Mendelian Inheritance in Man [30a]), also referred to as familial hyperinsulinism or, formerly, pancreatic nesidioblastosis, is an autosomal recessive disorder occurring in approximately 1/50,000 births in Western countries and 1/2,500 in some Arabic communities and is characterized by an excessive release of insulin, despite severe hypoglycemia (31,32). Histological features of PHHI were initially characterized as diffuse proliferation and budding of pancreatic islet cells from pancreatic ducts and often disorganized formation of new islets (33), hence "nesidioblastosis" (34), but quantitative immunohistochemical studies have shown that these features occur also in normal infants (35). Thus, PHHI or familial hyperinsulinism is now a more appropriate name for this syndrome. Linkage analysis suggested that PHHI was mapped to chromosome 11p14–11p15.1 (36). The SUR1 and Kir6.2 genes were found to be clustered at 11p15.1 with the Kir6.2 gene immediately 3′ of the SUR1 gene for ~4.5 kbp distance (16,37). The protein coding region of the SUR1 and Kir6.2 genes is composed of 39 exons (7) and a single exon (16), respectively. Recent studies have shown that impaired β-cell K_{ATP} channel function due to mutations of the SUR1 or Kir6.2 gene (38) is responsible for PHHI (39–41). The majority of the mutations are present in the SUR1 gene (38). To date, there are only two reports of mutations in the Kir6.2 gene (42,43). Thomas et al. (42) found a homozygous Kir6.2 gene mutation (L147P) in an affected individual with severe PHHI—a mutation that could result in a disruption of the M2 α-helical transmembrane domain of the protein. A homozygous nonsense mutation in the Kir6.2 gene, Y12X, was identified in a proband from a consanguineous Palestinian Arab family (43). Reconstitution from SUR1 and Kir6.2Y12X in COS-1 cells confirmed the absence of K_{ATP} channel activity (43). However, phenotypic differences between SUR1 and Kir6.2 mutations are not known at present.

MOUSE MODELS WITH IMPAIRED K_{ATP} CHANNEL FUNCTION

To elucidate the physiological roles of the K_{ATP} channels directly, we generated two mouse models: *1*) transgenic mice expressing a dominant-negative mutant of Kir6.2 in pancreatic β-cells (Kir6.2G132S Tg mice), and *2*) mice lacking Kir6.2 (Kir6.2 knockout mice).

Characteristics of transgenic mice expressing Kir6.2G132S in pancreatic β-cells. The K^+ ion-permeable domain H5 is highly conserved in K^+ channels, and the motif Gly-Tyr (or Phe)-Gly is thought to be critical for K^+ ion selectivity (13). A substitution of the first residue of the Gly-Tyr-Gly motif with Ser (residue 156) is found in the G-protein–gated inward rectifier GIRK2 (Kir3.2) of the neurological mutant *weaver* mice (44). By analogy with the *weaver* mutant, we replaced the residue 132 Gly with Ser (Kir6.2G132S mutant). Kir6.2G132S, when coexpressed with wild-type Kir6.2 and SUR1 in COS-1 cells, was shown to act as a dominant-negative inhibitor of K_{ATP} channels. The transgenic mice (Kir6.2G132S Tg mice) were generated only for the purpose of disruption of K_{ATP} channel function in pancreatic β-cells *1*) by using an insulin gene promoter to confine transgene expression to pancreatic β-cells and *2*) by the introduction of a dominant-negative mutant Kir6.2 (13). The K_{ATP} channel currents of the β-cells of the transgenic mice are significantly decreased. The resting membrane potential and basal intracellular calcium concentration ($[Ca^{2+}]_i$) of the β-cells of transgenic mice are significantly higher than those of control mice. Neonatal transgenic mice exhibit relatively high levels of serum insulin despite hypoglycemia, indicating that the phenotype resembles PHHI in humans (see the following section on Kir6.2 knockout mice); however, the transgenic mice develop hyperglycemia as adults. The degree of hyperglycemia varies from mild to severe among the different transgenic lines. Glucose-induced insulin secretion is markedly reduced in the transgenic mice with severe hyperglycemia. Histological analysis reveals abnormal architecture of the islets of transgenic mice. There is a marked decrease in the number of β-cells in adult transgenic mice. Apoptotic cells in the islets are detected at a higher frequency in transgenic mice. Interestingly, glucagon-positive α-cells, which are present in the periphery of the islets of normal mice, also appear in the central region in the islets of transgenic mice, probably because of acceleration of β-cell death.

Characteristics of Kir6.2 knockout mice. Because Kir6.2 subunits form the K^+ ion-selective pore of K_{ATP} channels, we disrupted the Kir6.2 gene by homologous recombination to generate mice lacking K_{ATP} channels (Kir6.2 knockout mice). Homozygous mice ($Kir6.2^{-/-}$ or Kir6.2 null mice [knockout mice hereafter]) were generated by interbreeding heterozygous mice ($Kir6.2^{+/-}$). K_{ATP} channel activity is completely absent in pancreatic β-cells, skeletal muscle, and heart of knockout mice, and these mice show a transient hypoglycemia as neonates similar to that of Kir6.2G132S Tg mice. Because loss of K_{ATP} channel function impairs glucose-dependent insulin secretion (see below), the insulin secretion at low concentrations of glucose is not suppressed and leads to hypoglycemia in Kir6.2 knockout mice and Kir6.2G132S Tg mice as neonates. This lack of suppression could account in part for the mechanism of hypoglycemia in PHHI. The action potentials are found already in the basal state (at low glucose) of the β-cells of knockout mice. The membrane potential is not hyperpolarized by the K^+ channel openers diazoxide and pinacidil. The basal $[Ca^{2+}]_i$ in β-cells of knockout mice is significantly elevated, but $[Ca^{2+}]_i$ in β-cells of knockout mice does not increase at all in response to either glucose or tolbutamide. In contrast, acetylcholine or high K^+ stimulation increases the $[Ca^{2+}]_i$ in the β-cells of knockout mice to levels comparable to control β-cells. Neither glucose at high concentrations nor tolbutamide elicits significant insulin secretion from the isolated islets of knockout mice. No glucose-dependent insulin secretion is found in the isolated islets of knockout mice. The insulin response to intraperitoneal glucose loading also is impaired but is clearly detected. Surprisingly, despite a severe defect in glucose-induced insulin secretion, glucose tolerance is found to be impaired only slightly in knockout mice. An insulin tolerance test shows that the glucose-lowering effect of insulin at a relatively low dose (0.1 U/kg) is significantly increased in knockout mice compared with controls. Histological examination reveals abnormal architecture of the islets, similar to that of the transgenic mice described above (glucagon-positive cells appear in the central region of the islets of the knockout mice). However, in contrast to Kir6.2G132S Tg mice, the number of β-cells and α-cells is only slightly decreased and significantly increased, respectively, in the knockout mice. Interestingly, the number of pancreatic polypeptide (PP)-positive cells (most of which coincide with glucagon-positive cells) is markedly increased in the islets of knockout mice.

Recently, SUR1 knockout mice ($SUR1^{-/-}$) have also been generated (45). A slight but significant glucose-induced insulin secretion is found in $SUR1^{-/-}$ mice. The reason for the difference in glucose-induced insulin secretion between Kir6.2 and SUR1 knockout mice is not known.

DIVERSE ROLES OF K_{ATP} CHANNELS IN ENDOCRINE PANCREAS LEARNED FROM GENETICALLY ENGINEERED MICE

Both glucose- and sulfonylurea-induced rises in $[Ca^{2+}]_i$ require closure of the K_{ATP} channels. K_{ATP} channels are known to be critical for determining the resting membrane potential of pancreatic β-cells as well as the depolarization induced by glucose (46–48). It is now accepted that closure of K_{ATP} channels causes β-cell membrane depolarization followed by opening of the voltage-dependent calcium channels (VDCCs), which allows calcium influx into β-cells (3). Studies of our transgenic mice and knockout mice provide direct evidence that K_{ATP} channels primarily determine the resting membrane potential and that closure of K_{ATP} channels is essential for glucose- and sulfonylurea-induced membrane depolarization. Furthermore, both the glucose- and sulfonylurea-induced rise in $[Ca^{2+}]_i$ can be initiated only by closure of the K_{ATP} channels. Because high K^+ stimulation increased $[Ca^{2+}]_i$ in the β-cells of the knockout mice, both the glucose- and sulfonylurea-induced rise in $[Ca^{2+}]_i$ are due to calcium influx through the VDCCs. In contrast, because acetylcholine stimulation increased $[Ca^{2+}]_i$ in β-cells of knockout mice, the intracellular calcium mobilization from inositol 1,4,5,triphosphate (IP_3)-sensitive Ca^{2+} stores occurs independently of closure of the K_{ATP} channels. However, both glucose and tolbutamide failed to increase $[Ca^{2+}]_i$ in β-cells of knockout mice, so the IP_3 pathway does not involve either the glucose- or sulfonylurea-induced rise in $[Ca^{2+}]_i$.

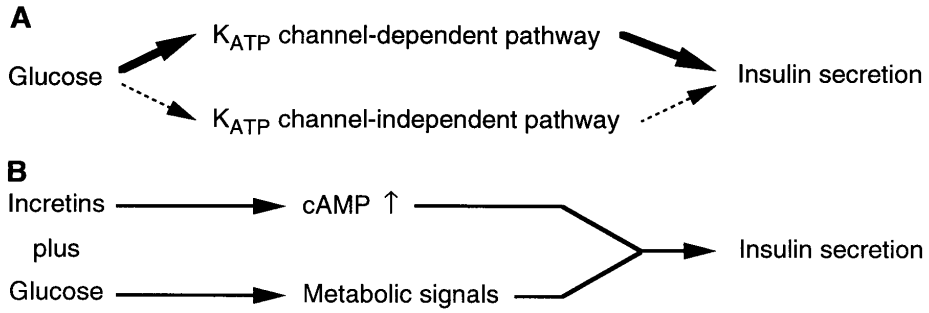

FIG. 2. Glucose-induced insulin secretion and its potentiation by incretins. *A*: Glucose-induced insulin secretion. The K_{ATP} channel–dependent pathway is the major pathway of glucose-induced insulin secretion. The contribution of the K_{ATP} channel–independent pathway to glucose-induced insulin secretion under physiological conditions is not known. *B*: Incretin-induced insulin secretion. cAMP is a major intracellular signal evoked by incretins. $[Ca^{2+}]_i$, cAMP, and metabolic signals all participate in the potentiation of insulin secretion by incretins. Incretin-induced insulin secretion is K_{ATP} channel–independent, and a rise in $[Ca^{2+}]_i$ is required for the effects.

Both glucose- and sulfonylurea-induced insulin secretions depend critically on the K_{ATP} channel-dependent pathway. Since the discovery of K_{ATP} channels in pancreatic β-cells (46–48), a model of glucose-induced insulin secretion via a K_{ATP} channel-dependent pathway has been proposed (3). In this model, the closure of K_{ATP} channels by glucose metabolism is essential for the rise in $[Ca^{2+}]_i$ that triggers insulin release (3). While this model has been generally accepted, Takasawa et al. (49) have proposed that cyclic ADP-ribose is a major intracellular signal for glucose-induced insulin secretion, the mechanism of which is independent of closure of the K_{ATP} channels in pancreatic β-cells. According to their model, cyclic ADP-ribose induced by glucose elicits intracellular calcium mobilization from a ryanodine-sensitive calcium pool. However, several studies show evidence against this model (50–52).

Aizawa and colleagues (53,54) and Henquin and colleagues (55,56) have recently proposed by pharmacological approaches that, in addition to a K_{ATP} channel–dependent mechanism, glucose simulates insulin secretion via a K_{ATP} channel–independent mechanism. However, our batch incubation and perifusion experiments on isolated pancreatic islets from Kir6.2G132S Tg mice and Kir6.2 knockout mice show clearly that glucose-induced insulin secretion depends critically on the activity of the K_{ATP} channels in pancreatic β-cells and that, regardless of any glucose-induced metabolic signals such as ATP and presumably glutamate, which has recently been identified (57), if the closure of the K_{ATP} channels does not occur, glucose cannot stimulate insulin secretion (Fig. 2*A*).

Tolbutamide-induced insulin secretion is absent in Kir6.2 knockout mice, indicating that sulfonylurea-induced insulin secretion also depends critically on activity of the K_{ATP} channels. Although it has recently been proposed that sulfonylureas might stimulate insulin secretion by acting directly on the sulfonylurea receptors, which constitute a functional part of the regulatory exocytotic protein (58), whether or not Kir6.2 also constitutes a functional part of such a protein is currently unknown.

Incretin-induced insulin secretion is mediated by the K_{ATP} channel–independent pathway. Despite the severe defect in glucose-induced insulin secretion, only a slight impairment in glucose intolerance is found in Kir6.2 knockout mice. Blood glucose levels in random measurements were not different between the Kir6.2 knockout mice and control mice until 20 weeks of age, when both were fed normal diet (S.S., T.M., unpublished observations). As described earlier, although there is almost no glucose-induced insulin secretion in the isolated islets, the insulin response to intraperitoneal glucose loading is slightly but clearly detected in knockout mice. In addition, the insulin response to meal ingestion is evident at 60 min after ingestion in knockout mice (S.S., T.M., unpublished observations). These findings suggest that mixed-meal-induced insulin secretion, most likely due to potentiating effects of incretins such as glucagon-like peptide 1 (GLP-1) and gastric inhibitory peptide (GIP), is retained in Kir6.2 knockout mice. Because intracellular cAMP induced by incretins and metabolic signals generated by glucose are both important in potentiation of insulin secretion by incretins (59) (Fig. 2*B*), elucidation of the relationship between cAMP signaling and glucose signaling in Kir6.2 knockout mice should provide clues to the mechanism of such potentiation. The elevation of $[Ca^{2+}]_i$ in the β-cells is a prerequisite for the effects of incretins (60). Although the elevation of $[Ca^{2+}]_i$ depends on activity of the K_{ATP} channels that are controlled by glucose concentrations, incretin-induced insulin secretion appears to be independent of the K_{ATP} channels.

K_{ATP} channels participate in glucose sensing in various glucose-responsive cells. We have found recently that recovery from hypoglycemia induced by exogenous insulin administration is markedly impaired in Kir6.2 knockout mice because of a defect in glucagon secretion (S.S., T.M., unpublished observations). It has been thought that the VMH plays a central role in the counterregulatory response to hypoglycemia (61). K_{ATP} channels are present in a certain population of neurons in the VMH (62). In situ hybridization studies show that Kir6.2 mRNA is expressed in the VMH (20). The VMH contains specific subsets of neurons that selectively alter their firing rate in response to changes in circulating glucose levels (63). These glucose-responsive neurons alter their firing rate as blood glucose levels change (64), a response that is mediated by K_{ATP} channels (62). We have found that glucagon response to high or low glucose in isolated islets from Kir6.2 knockout mice is not impaired (S.S., T.M., unpublished observations). Accordingly, it is possible that disruption of Kir6.2 impairs glucose sensing in glucose-responsive neurons in the VMH and leads to a defect in the glucagon response to hypoglycemia. In addition, K_{ATP} channels also exist in glucose-responsive enteric neurons, where they are suggested to play a role in gastrointestinal motility (21).

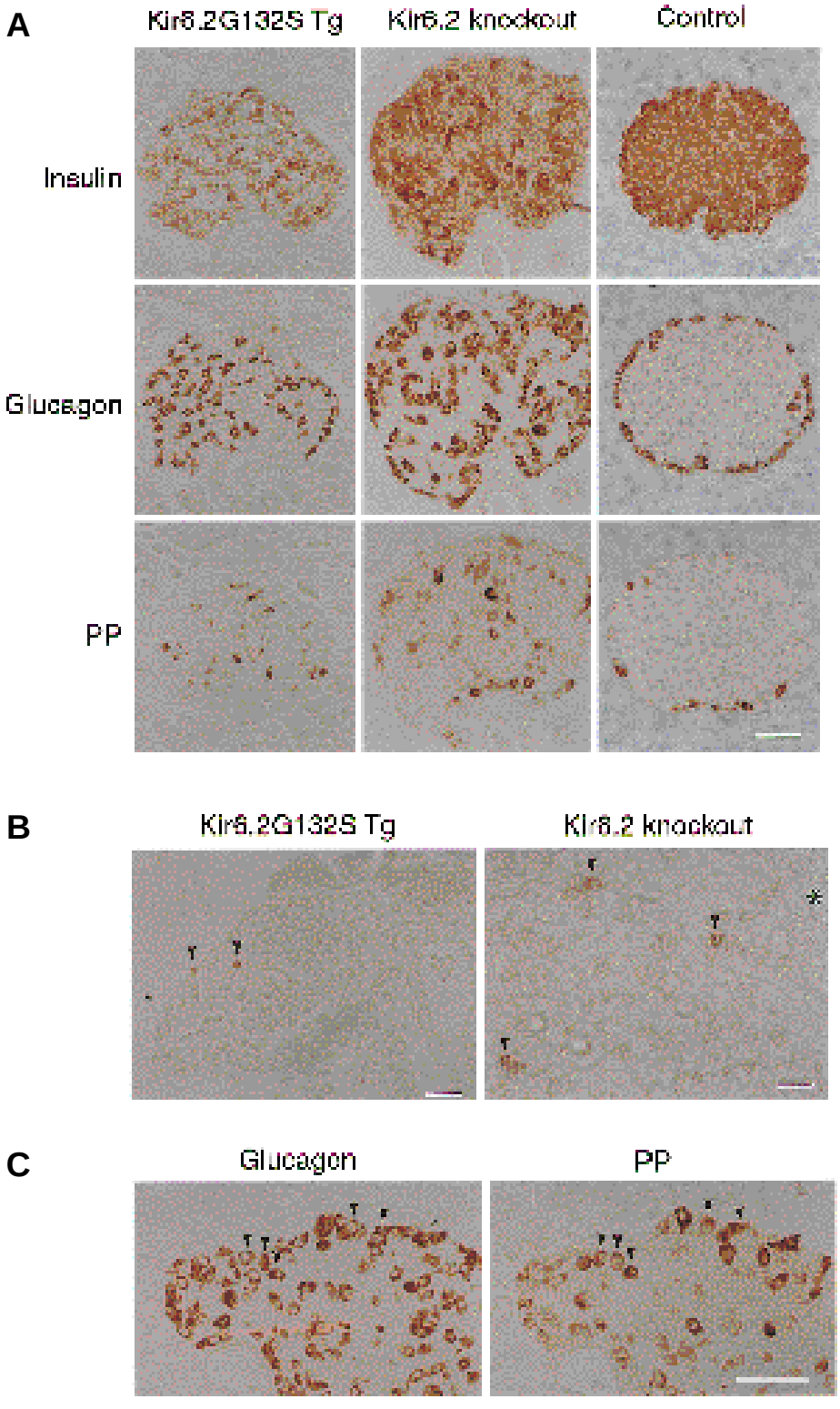

FIG. 3. Morphological changes in pancreatic islets of Kir6.2G132S Tg mice and Kir6.2 knockout mice. *A*: Insulin-, glucagon-, and PP-positive cells in pancreatic islets. Pancreatic islets of Kir6.2G132S Tg mice (10 weeks), Kir6.2 knockout mice (70 weeks), and control (wild-type) mice (90 weeks) were immunostained with anti-insulin, anti-glucagon, and anti-PP antibodies, respectively. The number of glucagon-positive cells is increased and they are scattered in the islets in both Kir6.2G132S Tg mice and Kir6.2 knockout mice. The number of PP-positive cells is increased in Kir6.2 knockout mice but not in Kir6.2G132S Tg mice. Somatostatin-positive δ-cells are not changed in topographical arrangement or cell number in Kir6.2 knockout mice (data not shown). Bar = 50 µm. *B*: Apoptosis in the islets of Kir6.2G132S Tg mice and Kir6.2 knockout mice. Apoptotic cells (arrowheads) are detected at a relatively high frequency in both Kir6.2G132S Tg mice (2 weeks) and Kir6.2 knockout mice (40 weeks) but are rarely detected in the respective control islets (not shown). *Exocrine pancreas. Bars = 20 µm (Kir6.2G132S Tg) and 10 µm (Kir6.2 knockout). *C*: Comparison of glucagon- and PP-positive cells in the islets of Kir6.2 knockout mice. Most PP-positive cells are coincident with glucagon-positive cells, as assessed by immunostaining serial sections (3-µm thickness) of the islets of a Kir6.2 knockout mouse (60 weeks). Arrowheads indicate typical cells that express both glucagon and PP. Bar = 50 µm.

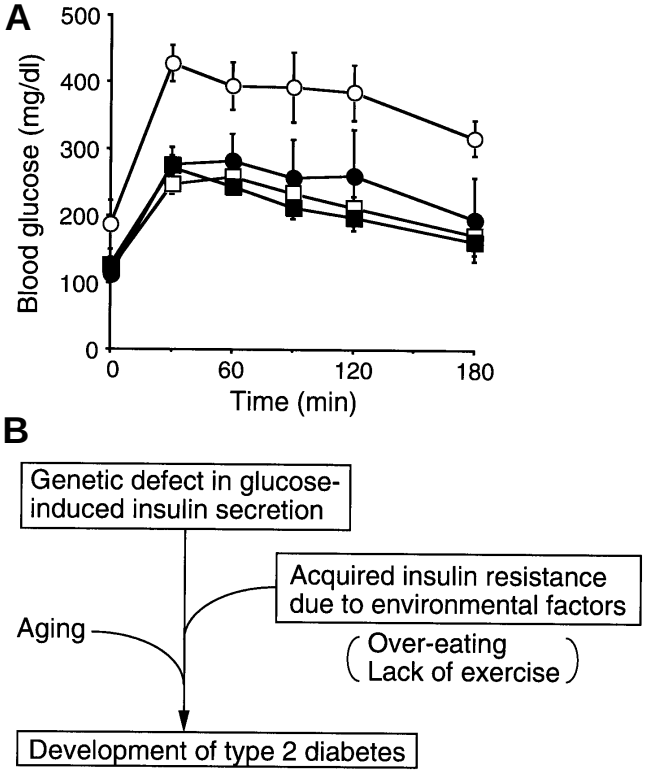

FIG. 4. A model of the development of type 2 diabetes learned from Kir6.2 knockout mice. *A*: Oral glucose tolerance test in aged Kir6.2 knockout mice and control mice. Fasting hyperglycemia and glucose intolerance are present in aged (55–60 weeks) obese Kir6.2 knockout mice, whereas glucose intolerance is not present in either age-matched nonobese Kir6.2 knockout mice or age-matched obese control mice. ○, Obese Kir6.2 knockout mice; □, obese control mice; ●, nonobese Kir6.2 knockout mice; ■, nonobese control mice. Changes in blood glucose levels (mean ± SE) after oral glucose loading (1 g/kg) are shown. *B*: A model for the development of type 2 diabetes. Both a genetic defect in glucose-induced insulin secretion and acquired insulin resistance due to environmental factors such as overeating and lack of exercise contribute to the development of type 2 diabetes. Aging is also a contributing factor.

Considering these findings, the K_{ATP} channels in glucose-responsive cells most probably participate in glucose sensing. K_{ATP} channels are important for β-cell survival and differentiation of islet cells. During the neonatal period, the morphology of the pancreatic islets of both Kir6.2G132S Tg mice and Kir6.2 knockout mice is normal. However, as they grow, the number of β-cells markedly and slightly decreases in the Tg mice and the knockout mice, respectively (Fig. 3*A*). By the TdT-mediated dUTP nick endo-labeling (TUNEL) method, apoptotic cells were detected both in Kir6.2G132S Tg mice and Kir6.2 knockout mice (Fig. 3*B*). In *weaver* mice, the mutation of the first Gly to Ser in the Gly-Tyr-Gly motif of the H5 region of Kir3.2 causes loss of ion selectivity, leading to chronic membrane depolarization of granule cells in the cerebellum (44). This chronic membrane depolarization has been thought to be responsible for the apoptotic cell death seen in granule cells in the cerebellum of *weaver* mice (65–67). Similarly, K_{ATP} channels appear to be important for β-cell survival by controlling its membrane potential.

Another interesting feature is that the number of PP-positive cells is not changed in Kir6.2G132S Tg mice, while it is markedly increased in Kir6.2 knockout mice. Staining serial sections of pancreatic islets with antibodies specific for PP and glucagon revealed that while the PP-positive cells do not coincide with the glucagon-positive cells in the Tg mice, most PP-positive cells are coincident with the glucagon-positive cells in the knockout mice (Fig. 3*C*). The PP-positive cells were not stained by anti–neuropeptide Y (NPY) antibodies (T.I., unpublished observations). It has been reported that although α-cells express neuropeptide Y during their development, glucagon and PP are not coexpressed in the same pancreatic cells during their development (68). In addition, by destroying precursor cells of the pancreatic islet cells using diphtheria toxin, Herrera et al. (69) reported that PP gene–expressing cells are indispensable for differentiation of β- and δ-cells, but not α-cells. However, our data on Kir6.2 knockout mice indicate that the PP gene can be expressed in α-cells or the glucagon gene can be expressed in PP-cells because of the disruption of the Kir6.2 gene in these cells. The coexpression of glucagon and PP in an islet cell in knockout mice suggests that the α-cell and PP-cell may have a common precursor cell with the ability to differentiate into either type of cell and that the K_{ATP} channels may play an important role in determining this differentiation. Disruption of the Kir6.2 gene could impair the terminal differentiation into α- or PP-cells. This hypothesis is compatible with a previous finding that the regional population of α- and PP-cells differs greatly and that they appear in inverse proportion to each other in human and canine pancreas (71,72). Gersell et al. (71) reported that α-cells are abundant in the body and tail of the pancreas, while PP-cells are abundant in the uncinate process and head. Kir6.2 knockout mice, therefore, may provide clues for a cell lineage of pancreatic islet cells.

A model of the development of type 2 diabetes and the clinical implications. When Kir6.2 knockout mice and control mice were fed normal diet (mixed meal), blood glucose levels at random measurements and body weight were not different between the knockout mice and the control mice until 20 weeks of age. Because insulin response to mixed-meal ingestion is retained in the knockout mice, incretin-induced insulin secretion via normal diet containing various nutrients may have prevented the development of hyperglycemia in these mice. Generally, the body weight of the mice maintained with one per cage on normal diet became significantly higher than that of the mice maintained with several mice per cage (Fig. 4*A*). Such knockout mice indeed became obese, as was found in control mice. Fasting blood glucose levels were significantly elevated in the aged Kir6.2 knockout mice with obesity, and glucose intolerance became evident. However, neither age-matched knockout mice without obesity nor age-matched control mice with obesity exhibited glucose intolerance. Although there is no direct evidence that mutations of the Kir6.2 or SUR1 gene cause diabetes, Kir6.2 knockout mice provide several clinically important suggestions regarding the pathophysiology and pathogenesis of type 2 diabetes (Fig. 4*B*). First, despite the presence of the severe defect in glucose-induced insulin secretion, if the potentiation mechanism, i.e., the incretin-induced insulin secretion, is intact, a mixed-meal diet could prevent the development of hyperglycemia by the secretion of insulin via the potentiation pathway. Second, if the potentiation mechanism is intact, a mixed-meal diet can cause obesity under certain environmental conditions, even in the presence of a severe defect in glucose-induced insulin secretion. Third, although insulin

resistance has been emphasized as a primary defect in the development of type 2 diabetes in Western countries, especially in the U.S. (73,74), the study of Kir6.2 knockout mice provides another model of the development of type 2 diabetes in which a genetic (primary) defect in insulin secretion and the acquired (secondary) insulin resistance due to environmental factors contribute to the development of type 2 diabetes. This model might be relevant to diabetes in Asian countries, where type 2 diabetes is commonly observed to be less related to insulin resistance or obesity than in the U.S., but rather to be related to impaired insulin secretion (75–80). Recent studies have shown that mice lacking the receptor for incretin, GLP-1, or GIP, which also exhibit impaired insulin secretion, develop glucose intolerance without the presence of insulin resistance (81,82). In GIP receptor knockout mice, postprandial hyperglycemia becomes evident when the mice develop insulin resistance caused by a high-fat diet (82). Incretin receptor knockout mice also suggest a model in which both a primary defect in insulin secretion and secondary insulin resistance contribute to the development of type 2 diabetes. Finally, it is clinically important to test not only insulin response to glucose loading, but also insulin response to mixed-meal ingestion, to evaluate the insulin secretory ability of patients and, therefore, to predict susceptibility of developing type 2 diabetes.

CONCLUSIONS

Studies of Kir6.2 genetically engineered mice have clarified various roles of the K_{ATP} channels in the endocrine pancreas, including glucose- and sulfonylurea-induced insulin secretion and islet cell differentiation. These studies also provide clinical implications on the pathophysiology and pathogenesis of type 2 diabetes. Because K_{ATP} channels are also present in various glucose-responsive cells other than pancreatic β-cells, understanding the regulatory mechanisms and functions of K_{ATP} channels should provide a better understanding of the physiological and pathophysiological roles of K_{ATP} channels in glucose homeostasis and its disorders and lead to the development of novel therapeutic agents for various diseases.

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REFERENCES

1. Ashcroft FM, Rorsman P: Electrophysiology of the pancreatic beta-cell. *Prog Biophys Mol Biol* 54:87–143, 1989
2. Meglasson MD, Matschinsky FM: Pancreatic islet glucose metabolism and regulation of insulin secretion. *Diabetes Metab Rev* 2:163–214, 1986
3. Cook DL, Satin LS, Ashford ML, Hales CN: ATP-sensitive K^+ channels in pancreatic beta-cells: spare-channel hypothesis. *Diabetes* 37:495–498, 1988
4. Misler S, Barnett DW, Gillis KD, Pressel DM: Electrophysiology of stimulus-secretion coupling in human beta-cells. *Diabetes* 41:1221–1228, 1992
5. Noma A: ATP-regulated K^+ channels in cardiac muscle. *Nature* 305:147–148, 1983
6. Ashcroft FM: Adenosine 5′-triphosphate-sensitive potassium channels. *Ann Rev Neurosci* 11:97–118, 1988
7. Aguilar-Bryan L, Clement JP IV, Gonzalez G, Kunjilwar K, Babenko A, Bryan J: Toward understanding the assembly and structure of K_{ATP} channels. *Physiol Rev* 78:227–245, 1998
8. Ashcroft FM, Gribble FM: Correlating structure and function in ATP-sensitive K^+ channels. *Trends Neurosci* 21:288–294, 1998
9. Seino S: ATP-sensitive potassium channels: a model of heteromultimeric potassium channel/receptor assemblies. *Annu Rev Physiol* 61:337–362, 1999
10. Amoroso S, Schmid-Antomarchi H, Fosset M, Lazdunski M: Glucose, sulfonylureas, and neurotransmitter release: role of ATP-sensitive K^+ channels. *Science* 247:852–854, 1990
11. Cole WC, McPherson CD, Sontag D: ATP-regulated K^+ channels protect the myocardium against ischemia/reperfusion damage. *Circ Res* 69:571–581, 1991
12. Heurteaux C, Lauritzen I, Widmann C, Lazdunski M: Essential role of adenosine, adenosine A1 receptors, and ATP-sensitive K^+ channels in cerebral ischemic preconditioning. *Proc Natl Acad Sci U S A* 92:4666–4670, 1995
13. Miki T, Tashiro F, Iwanaga T, Nagashima K, Yoshitomi H, Aihara H, Nitta Y, Gonoi T, Inagaki N, Miyazaki JI, Seino S: Abnormalities of pancreatic islets by targeted expression of a dominant-negative K_{ATP} channel. *Proc Natl Acad Sci U S A* 94:11969–11973, 1997
14. Miki T, Nagashima K, Tashiro F, Kotake K, Yoshitomi H, Tamamoto A, Gonoi T, Iwanaga T, Miyazaki JI, Seino S: Defective insulin secretion and enhanced insulin action in K_{ATP} channel-deficient mice. *Proc Natl Acad Sci U S A* 95:10402–10406, 1998
15. Inagaki N, Tsuura Y, Namba N, Masuda K, Gonoi T, Horie M, Seino Y, Mizuta M, Seino S: Cloning and functional characterization of a novel ATP-sensitive potassium channel ubiquitously expressed in rat tissues, including pancreatic islets, pituitary, skeletal muscle, and heart. *J Biol Chem* 270:5691–5694, 1995
16. Inagaki N, Gonoi T, Clement JP IV, Namba N, Inazawa J, Gonzalez G, Aguilar-Bryan L, Seino S, Bryan J: Reconstitution of IKATP: an inward rectifier subunit plus the sulfonylurea receptor. *Science* 270:1166–1170, 1995
17. Suzuki M, Fujikura K, Inagaki N, Seino S, Takata K: Localization of the ATP-sensitive K^+ channel subunit Kir6.2 in mouse pancreas. *Diabetes* 46:1440–1444, 1997
18. Suzuki M, Fujikura K, Kotake K, Inagaki N, Seino S, Takata K: Immuno-localization of sulphonylurea receptor 1 in rat pancreas. *Diabetologia* 42:1204–1211, 1999
19. Karschin C, Ecke C, Ashcroft FM, Karschin A: Overlapping distribution of K_{ATP} channel-forming Kir6.2 subunit and the sulfonylurea receptor SUR1 in rodent brain. *FEBS Lett* 401:59–64, 1997
20. Dunn-Meynell AA, Rawson NE, Levin BE: Distribution and phenotype of neurons containing the ATP-sensitive K^+ channel in rat brain. *Brain Res* 814:41–54, 1998
21. Liu M-T, Seino S, Kirchgessner AL: Identification and characterization of glucoresponsive neurons in the enteric nervous system. *J Neurosci* 19:10305–10317, 1999
22. Clement JP, Kunjilwar K, Gozalez G, Schwanstecher M, Peanten U, Aguilar-Bryan L, Bryan J: Association and stoichiometry of K_{ATP} channel subunits. *Neuron* 18:827–838, 1997
23. Inagaki N, Gonoi T, Seino S: Subunit stoichiometry of the pancreatic β-cell K^+ channel. *FEBS Lett* 409:232–236, 1997
24. Shyng S, Nichols CG: Octameric stoichiometry of the K_{ATP} channel complex. *J Gen Physiol* 110:655–664, 1997
25. Tucker SJ, Gribble FM, Zhao C, Trapp S, Ashcroft FM: Truncation of Kir6.2 produces ATP-sensitive K^+ channels in the absence of the sulphonylurea receptor. *Nature* 387:179–183, 1997
26. Zerangue N, Schwappach B, Jan YN, Jan LY: A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane K_{ATP} channels. *Neuron* 22:537–548, 1999
27. Ueda K, Inagaki N, Seino S: MgADP antagonism to Mg^{2+}-independent ATP binding of the sulfonylurea receptor SUR1. *J Biol Chem* 272:22983–22986, 1997
28. Shyng S, Ferrigni T, Nichols CG: Regulation of KATP channel activity by diazoxide and MgADP: distinct functions of the two nucleotide binding folds of the sulfonylurea receptor. *J Gen Physiol* 110:643–654, 1997
29. Gribble FM, Tucker SJ, Haug T, Ashcroft FM: MgATP activates the β-cell K_{ATP} channel by interaction with its SUR1 subunit. *Proc Natl Acad Sci U S A* 95:7185–7190, 1998
30. Béguin P, Nagashima K, Nishimura M, Gonoi T, Seino S: PKA-mediated phosphorylation of the human K_{ATP} channel: separate roles of Kir6.2 and SUR1 subunit phosphorylation. *EMBO J* 18:4722–4732, 1999

30a. Online Mendelian Inheritance in Man: http://www.ncbi.nlm.nih.gov/omim

31. Permutt MA, Nestrowicz A, Glaser B: Familial hyperinsulinism: an inherited

disorder of spontaneous hypoglycemia in neonates and infants. *Diabetes Rev* 4:347–355, 1996
32. Aguilar-Bryan L, Bryan J: ATP-sensitive potassium channels, sulfonylurea receptors, and persistent hyperinsulinemic hypoglycemia of infancy. *Diabetes Rev* 4:336–346, 1996
33. Yakovac WC, Baker L, Hummeler K: Beta cell nesidioblastosis in idiopathic hypoglycemia of infancy. *J Pediatr* 79:226–231, 1971
34. Laidlaw GF: Nesidioblastoma: the islet tumor of the pancreas. *Am J Pathol* 14:125–134, 1938
35. Heitz PU, Kloppel G, Hacki WH, Polak JM, Pearse AG: Nesidioblastosis: the pathologic basis of persistent hyperinsulinemic hypoglycemia in infants: morphologic and quantitative analysis of seven cases based on specific immunostaining and electron microscopy. *Diabetes* 26:632–642, 1977
36. Glaser B, Chiu KC, Anker R, Nestorowicz A, Landau H, Ben-Bassat H, Shlomai Z, Kaiser N, Thornton PS, Stanley CA, Spielman RS, Gogolin-Ewens K, Cerasi E, Baker L, Donis-Keller H, Permutt MA: Familial hyperinsulinism maps to chromosome 11p14–15.1, 30 cM centromeric to the insulin gene. *Nat Genet* 7:185–188, 1994
37. Aguilar-Bryan L, Nichols CG, Wechsler SW, Clement JP IV, Boyd AE III, Gonzalez G, Herrera-Sosa H, Nguy K, Bryan J, Nelson DA: Cloning of the β cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. *Science* 268:423–426, 1995
38. Aguilar-Bryan L, Bryan J: Molecular biology of adenosine triphosphate-sensitive potassium channels. *Endocr Rev* 20:101–135, 1999
39. Kane C, Shepherd RM, Squires PE, Johnson PR, James RF, Milla PJ, Aynsley-Green A, Lindley KJ, Dunne MJ: Loss of functional K_{ATP} channels in pancreatic beta-cells causes persistent hyperinsulinemic hypoglycemia of infancy. *Nat Med* 2:1344–1347, 1996
40. Nichols CG, Shyng SL, Nestorowicz A, Glaser B, Clement JP IV, Gonzalez G, Aguilar-Bryan L, Permutt MA, Bryan J: Adenosine diphosphate as an intracellular regulator of insulin secretion. *Science* 272:1785–1787, 1996
41. Dunne MJ, Kane C, Shepherd RM, Sanchez JA, James RF, Johnson PR, Aynsley-Green A, Lu S, Clement JP IV, Lindley KJ, Seino S, Aguilar-Bryan L: Familial persistent hyperinsulinemic hypoglycemia of infancy and mutations in the sulfonylurea receptor. *N Engl J Med* 336:703–706, 1997
42. Thomas PM, Ye Y, Lightner E: Mutation of the pancreatic islet inward rectifier Kir6.2 also leads to familial persistent hyperinsulinemic hypoglycemia of infancy. *Hum Mol Genet* 5:1809–1812, 1996
43. Nestrowitz A, Inagaki N, Gonoi T, Schoor KP, Wilson BA, Glaser B, Landau H, Stanley CA, Thorenton PS, Seino S, Permutt A: A nonsense mutation in the inward rectifier potassium channel gene, Kir6.2, is associated with familial hyperinsulinism. *Diabetes* 46:1743–1748, 1997
44. Patil N, Cox DR, Bhat D, Faham M, Myers RM, Peterson AS: A potassium channel mutation in weaver mice implicates membrane excitability in granule cell differentiation. *Nat Genet* 11:126–129, 1995
45. Seghers VJ, Nakazaki M, Gonzalez G, Demaya FJ, Aguilar-Bryan L, Bryan J: Analysis of K_{ATP} channel deficient $SUR^{-/-}$ mice (Abstract). *Diabetes* (Suppl. 1):A34, 1999
46. Ashcroft FM, Harrison DE, Ashcroft SJH: Glucose induces closure of single potassium channels in isolated rat pancreatic β-cells. *Nature* 312:446–448, 1984
47. Cook DL, Hales CN: Intracellular ATP directly blocks K^+ channels in pancreatic β-cells. *Nature* 311:271–273, 1984
48. Rorsman P, Trube G: Glucose dependent K^+-channels in pancreatic β-cells are regulated by intracellular ATP. *Pflugers Arch* 405:305–309, 1985
49. Takasawa S, Nata K, Yonekura H, Okamoto H: Cyclic ADP-ribose in insulin secretion from pancreatic beta cells. *Science* 259:370–373, 1993
50. Islam MS, Larsson O, Berggren PO: Cyclic ADP-ribose in beta cells. *Science* 262:584–586, 1993
51. Rutter GA, Theler JM, Li G, Wollheim CB: Ca^{2+} stores in insulin-secreting cells: lack of effect of cADP ribose. *Cell Calcium* 16:71–80, 1994
52. Webb DL, Islam MS, Efanov AM, Brown G, Kohler M, Larsson O, Berggren PO: Insulin exocytosis and glucose-mediated increase in cytoplasmic free Ca^{2+} concentration in the pancreatic beta-cell are independent of cyclic ADP-ribose. *J Biol Chem* 271:19074–19079, 1996
53. Sato Y, Aizawa T, Komatsu M, Okada N, Yamada T: Dual functional role of membrane depolarization/Ca^{2+} influx in rat pancreatic B-cell. *Diabetes* 41:438–443, 1992
54. Aizawa T, Sato Y, Ishihara F, Taguchi N, Komatsu M, Suzuki N, Hashizume K, Yamada T: ATP-sensitive K^+ channel-independent glucose action in rat pancreatic beta-cell. *Am J Physiol* 266:C622–C627, 1994
55. Gembal M, Gilon P, Henquin JC: Evidence that glucose can control insulin release independently from its action on ATP-sensitive K^+ channels in mouse B cells. *J Clin Invest* 89:1288–1295, 1992
56. Gembal M, Detimary P, Gilon P, Gao ZY, Henquin JC: Mechanisms by which glucose can control insulin release independently from its action on adenosine triphosphate-sensitive K^+ channels in mouse B cells. *J Clin Invest* 91:871–880, 1993
57. Maechler P, Wollheim CB: Mitochondrial glutamate acts as a messenger in glucose-induced insulin exocytosis. *Nature* 402:685–689, 1999
58. Eliasson L, Renstrom E, Ammala C, Berggren PO, Bertorello AM, Bokvist K, Chibalin A, Deeney JT, Flatt PR, Gabel J, Gromada J, Larsson O, Lindstrom P, Rhodes CJ, Rorsman P: PKC-dependent stimulation of exocytosis by sulfonylureas in pancreatic beta cells. *Science* 271:813–815, 1996
59. Zawalich WS, Rasmussen H: Control of insulin secretion: a model involving Ca^{2+}, cAMP and diacylglycerol. *Mol Cell Endocrinol* 70:119–137, 1990
60. Sato Y, Nenquin M, Henquin JC: Relative contribution of Ca^{2+}-dependent and Ca^{2+}-independent mechanisms to the regulation of insulin secretion by glucose. *FEBS Lett* 421:115–119, 1998
61. Borg WP, During MJ, Sherwin RS, Borg MA, Brines ML, Shulman GI: Ventromedial hypothalamic lesions in rats suppress counterregulatory responses to hypoglycemia. *J Clin Invest* 93:1677–1682, 1994
62. Ashford ML, Boden PR, Treherne JM: Glucose-induced excitation of hypothalamic neurones is mediated by ATP-sensitive K^+ channels. *Pflügers Arch* 415:479–483, 1990
63. Minami T, Oomura Y, Sugimori M: Electrophysiological properties and glucose responsiveness of guinea-pig ventromedial hypothalamic neurones in vitro. *J Physiol (Lond)* 380:127–143, 1986
64. Levin BE, Dunn-Meynell AA, Routh VH: Brain glucose sensing and body energy homeostasis: role in obesity and diabetes. *Am J Physiol* 276:R1223–R1231, 1999
65. Slesinger PA, Patil N, Liao YJ, Jan YN, Jan LY, Cox DR: Functional effects of the mouse weaver mutation on G protein-gated inwardly rectifying K^+ channels. *Neuron* 16:321–331, 1996
66. Kofuji P, Hofer M, Millen KJ, Millonig JM, Davidson N, Lester HA, Hatten ME: Functional analysis of the weaver mutant GIRK2 K^+ channel and rescue of weaver granule cells. *Neuron* 16:941–952, 1996
67. Navarro B, Kennedy ME, Velimirovic B, Bhat D, Peterson AS, Clapham DE: Nonselective and G betagamma-insensitive weaver K^+ channels. *Science* 272: 1950–1953, 1996
68. Teitelman G, Alpert S, Polak JM, Martinez A, Hanahan D: Precursor cells of mouse endocrine pancreas coexpress insulin, glucagon and the neuronal proteins tyrosine hydroxylase and neuropeptide Y, but not pancreatic polypeptide. *Development* 118:1031–1039, 1993
69. Herrera PL, Huarte J, Zufferey R, Nichols A, Mermillod B, Philippe J, Muniesa P, Sanvito F, Orci L, Vassalli JD: Ablation of islet endocrine cells by targeted expression of hormone-promoter-driven toxigenes. *Proc Natl Acad Sci U S A* 91:12999–13003, 1994
71. Gersell DJ, Gingerich RL, Greider MH: Regional distribution and concentration of pancreatic polypeptide in the human and canine pancreas. *Diabetes* 28:11–15, 1979
72. Muranishi T, Takehana K, Hiratsuka T, Kobayashi A, Eerdunchaolu, Iwasa K, Abe M: An investigation of the relationship between duct system and A cell-rich and PP cell-rich pancreatic islets in the canine pancreas. *J Vet Med Sci* 61:737–742, 1999
73. Reaven GM: Role of insulin resistance in human disease. *Diabetes* 37:1595–1607, 1988
74. Martin BC, Warram JH, Krolewski AS, Bergman RN, Soeldner JS, Kahn CR: Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. *Lancet* 340:925–929, 1992
75. Seino Y, Ikeda M, Yawata M, Imura H: The insulinogenic index in secondary diabetes. *Horm Metab Res* 7:107–115, 1975
76. Seino Y, Taminato T, Goto Y, Imura H: Comparative insulinogenic effects of glucose, arginine and glucagon in patients with diabetes mellitus, endocrine disorders and liver diseases. *Acta Diabetol* 12:107–111, 1975
77. Kosaka K, Akanuma Y: Heterogeneity of plasma IRI responses in patients with IGT. *Diabetologia* 18:347–348, 1980
78. Min HK: Non-insulin-dependent diabetes mellitus (NIDDM) in Korea. *Diabet Med* 9 (Suppl. 6):S13–S15, 1996
79. Shin CS, Lee HK, Koh CS, Kim YI, Shin YS, Yoo KY, Paik HY, Park YS, Yang BG: Risk factors for the development of NIDDM in Yonchon County, Korea. *Diabetes Care* 12:1842–1846, 1997
80. Yoshinaga H, Kosaka K: Heterogeneous relationship of early insulin response and fasting insulin level with development of non-insulin-dependent diabetes mellitus in non-diabetic Japanese subjects with or without obesity. *Diabetes Res Clin Pract* 44:129–136, 1999
81. Scrocchi LA, Brown TJ, MaClusky N, Brubaker PL, Auerbach AB, Joyner AL, Drucker DJ: Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. *Nat Med* 11:1254–1258, 1996
82. Miyawaki K, Yamada Y, Yano H, Niwa H, Ban N, Ihara Y, Kubota A, Fujimoto S, Kajikawa M, Kurose A, Tsuda K, Hashimoto H, Yamashita T, Jomori T, Tashiro F, Miyazaki J-I, Seino Y: Glucose intolerance caused by a defect in the entero-insular axis: a study in gastric inhibitory polypeptide receptor knockout mice. *Proc Natl Acad Sci U S A* 96:14843–14847, 1999