

# Perspectives in Diabetes

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

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The regulation of insulin secretion from pancreatic  $\beta$ -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  $\beta$ -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  $\beta$ -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  $\beta$ -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  $\beta$ -cells, 2) both glucose- and sulfonylurea-induced membrane depolarization of  $\beta$ -cells require closure of the  $K_{ATP}$  channels, 3) both glucose- and sulfonylurea-induced rises in intracellular calcium concentration in  $\beta$ -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  $\beta$ -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

**P**ancreatic  $\beta$ -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  $\beta$ -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  $\beta$ -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  $\alpha$ -subunit, Kir6.2, and a sulfonylurea receptor  $\beta$ -subunit, SUR1 or SUR2 variant, that belongs to the ATP-binding cassette transporter superfamily (7–9). The pancreatic  $\beta$ -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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[ $Ca^{2+}$ ]<sub>i</sub>, 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  $\beta$ -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  $\beta$ -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 (Kir6.2 $\Delta$ C26 or Kir6.2 $\Delta$ C36) 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 (Kir6.2 $\Delta$ C26 or Kir6.2 $\Delta$ C36) 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  $\beta$ -cell  $K_{ATP}$  channels (30). On the other hand, SUR1 can be phosphorylated at Ser residue 1571, which is

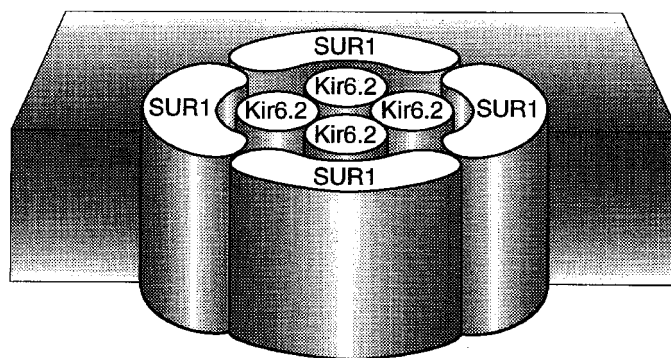


FIG. 1. Subunit assembly of pancreatic  $\beta$ -cell  $K_{ATP}$  channel. The  $\beta$ -cell  $K_{ATP}$  channel is a hetero-octamer of Kir6.2 and SUR1 with 4:4 stoichiometry.

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.

#### 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  $\beta$ -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  $\alpha$ -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  $\beta$ -cells (Kir6.2G132S Tg mice), and 2) mice lacking Kir6.2 (Kir6.2 knockout mice).

Characteristics of transgenic mice expressing Kir6.2G132S in pancreatic  $\beta$ -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  $\beta$ -cells 1) by using an insulin gene promoter to confine transgene expression to pancreatic  $\beta$ -cells and 2) by the introduction of a dominant-negative mutant Kir6.2 (13). The  $K_{ATP}$  channel currents of the  $\beta$ -cells of the transgenic mice are significantly decreased. The resting membrane potential and basal intracellular calcium concentration ( $[Ca^{2+}]_i$ ) of the  $\beta$ -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  $\beta$ -cells in adult transgenic mice. Apoptotic cells in the islets are detected at a higher frequency in transgenic mice. Interestingly, glucagon-positive  $\alpha$ -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  $\beta$ -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<sup>-/-</sup> or Kir6.2 null mice [knockout mice hereafter]) were generated by interbreeding heterozygous mice (Kir6.2<sup>+/-</sup>).  $K_{ATP}$  channel activity is completely absent in pancreatic  $\beta$ -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  $\beta$ -cells of knockout mice. The membrane potential is

not hyperpolarized by the  $K^+$  channel openers diazoxide and pinacidil. The basal  $[Ca^{2+}]_i$  in  $\beta$ -cells of knockout mice is significantly elevated, but  $[Ca^{2+}]_i$  in  $\beta$ -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  $\beta$ -cells of knockout mice to levels comparable to control  $\beta$ -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  $\beta$ -cells and  $\alpha$ -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<sup>-/-</sup>) have also been generated (45). A slight but significant glucose-induced insulin secretion is found in SUR1<sup>-/-</sup> 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  $\beta$ -cells as well as the depolarization induced by glucose (46–48). It is now accepted that closure of  $K_{ATP}$  channels causes  $\beta$ -cell membrane depolarization followed by opening of the voltage-dependent calcium channels (VDCCs), which allows calcium influx into  $\beta$ -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  $\beta$ -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  $\beta$ -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  $\beta$ -cells of knockout mice, so the  $IP_3$  pathway does not involve either the glucose- or sulfonylurea-induced rise in  $[Ca^{2+}]_i$ .

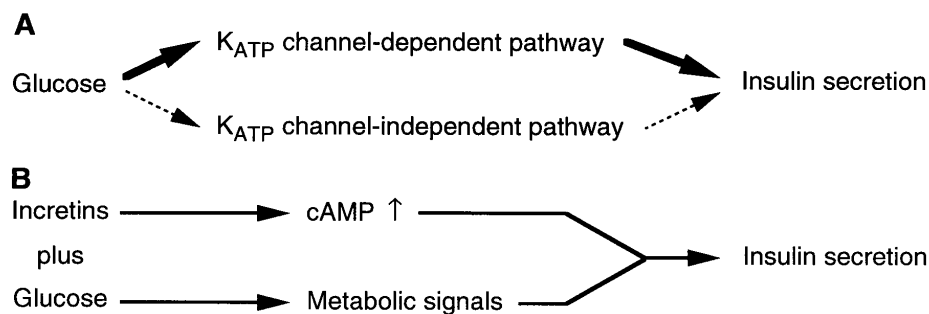


FIG. 2. Glucose-induced insulin secretion and its potentiation by incretins. A: Glucose-induced insulin secretion. The  $K_{ATP}$  channel-dependent pathway is the major pathway of glucose-induced insulin secretion. The contribution of the  $K_{ATP}$  channel-independent pathway to glucose-induced insulin 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  $\beta$ -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  $\beta$ -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).

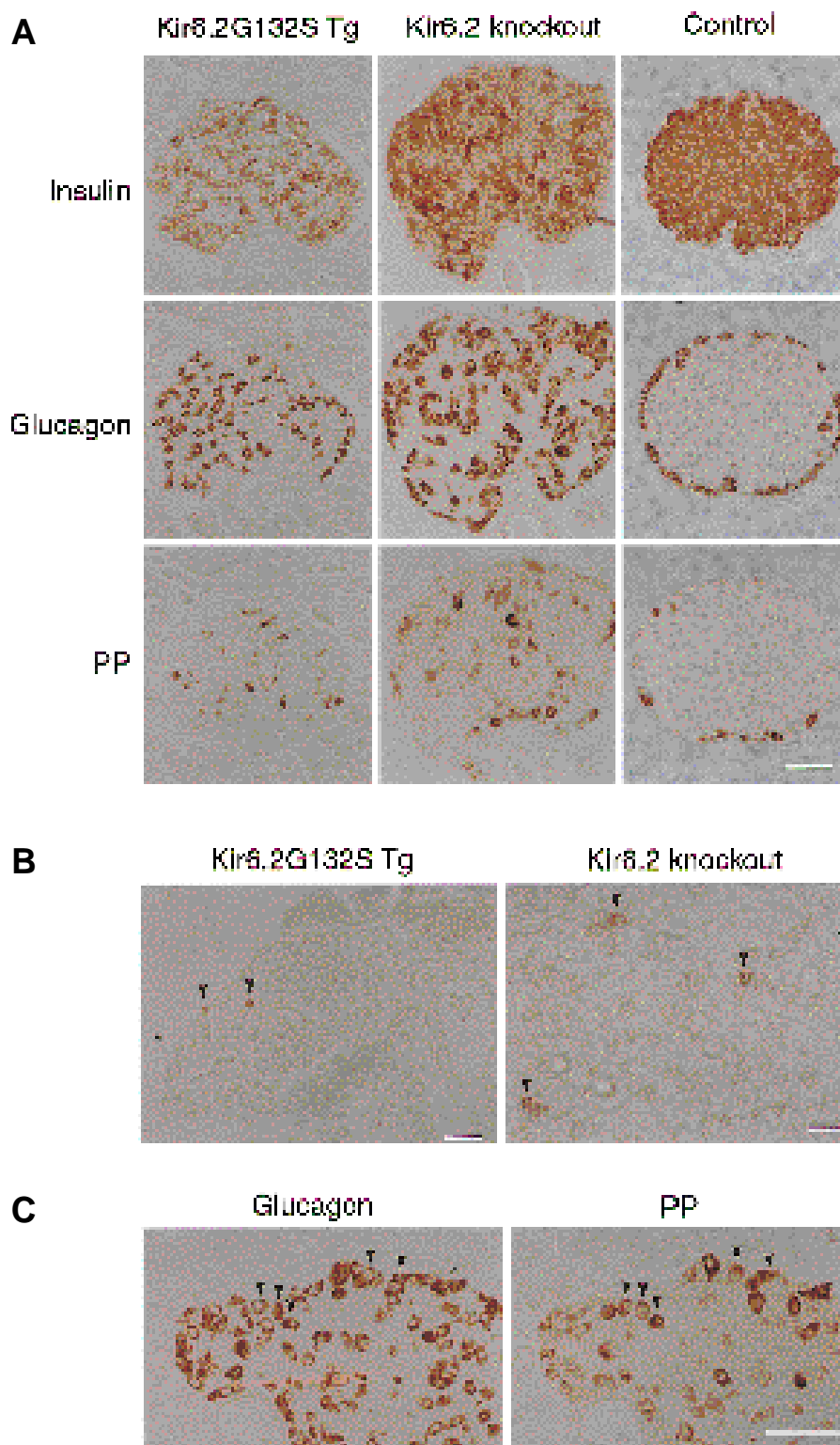
Aizawa and colleagues (53,54) and Henquin and colleagues (55,56) have recently proposed by pharmacological approaches that, in addition to a  $K_{ATP}$  channel-dependent mechanism, glucose stimulates insulin secretion via a  $K_{ATP}$  channel-independent mechanism. However, our batch incubation and perfusion 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  $\beta$ -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. 2A).

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. 2B), 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  $\beta$ -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  $\delta$ -cells are not changed in topographical arrangement or cell number in Kir6.2 knockout mice (data not shown). Bar = 50  $\mu$ m. **B:** Apoptosis in the islets of Kir6.2G132S Tg mice and Kir6.2 knockout mice (40 weeks) but are rarely detected in the respective control islets (not shown). \*Exocrine pancreas. Bars = 20  $\mu$ m (Kir6.2G132S Tg) and 10  $\mu$ m (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- $\mu$ m thickness) of the islets of a Kir6.2 knockout mouse (60 weeks). Arrowheads indicate typical cells that express both glucagon and PP. Bar = 50  $\mu$ m.

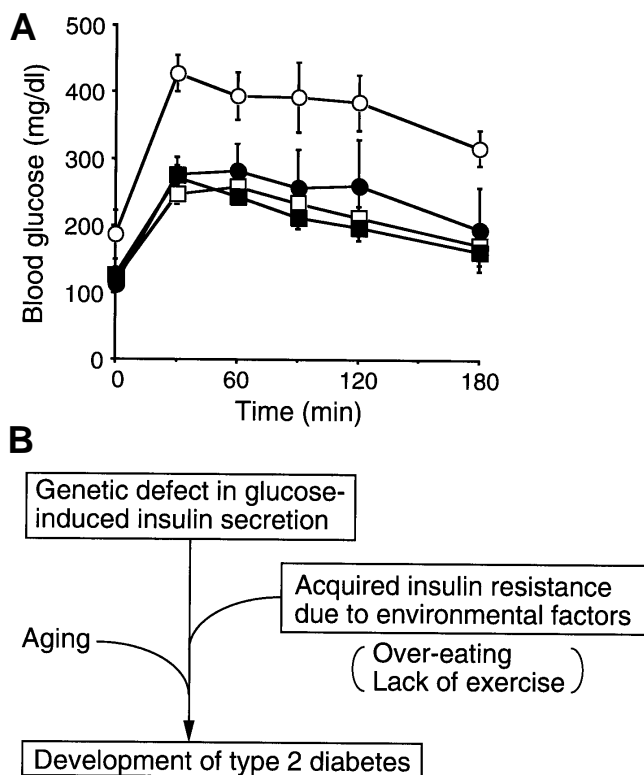


FIG. 4. A model of the development of type 2 diabetes learned from Kir6.2 knockout mice. A: Oral glucose tolerance test in aged Kir6.2 knockout mice and control mice. Fasting hyperglycemia and glucose intolerance are present in aged (55–60 weeks) obese Kir6.2 knockout mice, whereas glucose intolerance is not present in either age-matched nonobese Kir6.2 knockout mice or age-matched obese control mice.  $\circ$ , Obese Kir6.2 knockout mice;  $\square$ , obese control mice;  $\bullet$ , nonobese Kir6.2 knockout mice;  $\blacksquare$ , nonobese control mice. Changes in blood glucose levels (mean  $\pm$  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. Ageing 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  $\beta$ -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  $\beta$ -cells markedly and slightly decreases in the Tg mice and the knockout mice, respectively (Fig. 3A). 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. 3B). 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  $\beta$ -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. 3C). The PP-positive cells were not stained by anti-neuropeptide Y (NPY) antibodies (T.I., unpublished observations). It has been reported that although  $\alpha$ -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  $\beta$ - and  $\delta$ -cells, but not  $\alpha$ -cells. However, our data on Kir6.2 knockout mice indicate that the PP gene can be expressed in  $\alpha$ -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  $\alpha$ -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  $\alpha$ - or PP-cells. This hypothesis is compatible with a previous finding that the regional population of  $\alpha$ - 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  $\alpha$ -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. 4A). 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. 4B). 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  $\beta$ -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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