As the rate-limiting controller of glucose metabolism, glucokinase represents the primary β-cell “glucose sensor.” Inactivation of both glucokinase (GK) alleles results in permanent neonatal diabetes; inactivation of a single allele causes maturity-onset diabetes of the young type 2 (MODY-2). Similarly, mice lacking both alleles (GK−/−) exhibit severe neonatal diabetes and die within a week, whereas heterozygous GK+/− mice exhibit markedly impaired glucose tolerance and diabetes, resembling MODY-2. Glucose metabolism increases the cytosolic [ATP]-to-[ADP] ratio, which closes ATP-sensitive K⁺ channels (Kₐᵥ channels), leading to membrane depolarization, Ca²⁺ entry, and insulin exocytosis. Glucokinase insufficiency causes defective KATP channel regulation, which may underlie the impaired secretion. To test this prediction, we crossed mice lacking neuroendocrine glucokinase (nGK−/−) with mice lacking KATP channels (Kir6.2−/−). Kir6.2 knockout rescues perinatal lethality of nGK−/−, although nGK−/−Kir6.2−/− animals are postnatally diabetic and still die prematurely. nGK−/− animals are diabetic on the Kir6.2−/− background but only mildly glucose intolerant on the Kir6.2−/− background. In the presence of glutamine, isolated nGK−/−Kir6.2−/− islets show improved insulin secretion compared with nGK−/−Kir6.2+/+. The significant abrogation of nGK−/− and nGK−/− phenotypes in the absence of KATP demonstrate that a major factor in glucokinase deficiency is indeed altered KATP signaling. The results have implications for understanding and therapy of glucokinase-related diabetes. Diabetes 54:2925–2931, 2005

Glucose metabolism in pancreatic β-cells is necessary to stimulate insulin secretion (1). Glucokinase, which phosphorylates glucose in the first, rate-limiting reaction, is a key component in the secretory pathway (2,3), and mutations of the GK gene are diabeticogenic in humans (4). Heterozygous inactivating mutations cause maturity-onset diabetes of the young type 2 (MODY-2), an autosomal, dominantly inherited form of diabetes characterized by an early age of onset and pancreatic β-cell dysfunction (5–11). Homozygous inactivating GK mutations cause permanent neonatal diabetes in humans (12,13), which is characterized by perinatal hyperglycemia and low birth weight (14). The disease usually requires insulin treatment throughout the patient’s lifetime, starting within the first month of life. Targeted disruption of the neuroendocrine knockout of GK (nGK) gene in mice also causes diabetes (15). These mice lack glucokinase in pancreatic β-cells and neurons but maintain normal liver glucokinase activity. Heterozygous null mice show early-onset mild diabetes caused by an impaired insulin-secretory response to glucose, resembling MODY-2. Importantly, homozygous nGK-deficient mice show severe perinatal diabetes, as in permanent neonatal diabetes, and die within a few days of birth (15).

ATP-sensitive K⁺ channels (Kₐᵥ channels) couple glucose metabolism to cellular electrical activity and therefore play a critical role in excitation-secretion coupling in the pancreatic β-cell. Glucose metabolism raises the cytosolic [ATP]-to-[ADP] ratio, which closes the Kₐᵥ channel and depolarization of the β-cell membrane. Membrane depolarization, in turn, leads to opening of voltage-dependent Ca²⁺ channels and a rise in intracellular [Ca²⁺], which triggers insulin vesicle exocytosis (16) (Fig. 1). Glucose metabolism has additional downstream actions, including generation of GTP, which may activate "Kₐᵥ-independent" pathways of insulin secretion. Namely, both electrical triggering of secretion through Kₐᵥ channels and nonelectrical effects of glucose metabolism through ATP generation could be affected by glucokinase deficiency. The relative importance of each component is not clear, and it might be presumed that the consequences of failure to generate ATP should be much more devastating than simply blocking electrical signaling.

It has previously been shown that glucose fails to close Kₐᵥ channels in nGK−/− β-cells (17). Sulfonylureas act by closing the Kₐᵥ channel independently of glucose metabolism, resulting in membrane depolarization and consequent stimulation of insulin secretion (18). Consistent with the idea that the primary downstream consequence of nGK deficiency is simply failure to close Kₐᵥ channels, the sulfonylurea tolbutamide was effective at both inhibiting Kₐᵥ channels and stimulating insulin secretion in nGK−/− β-cells (17). These results imply that the defect in insulin secretion in glucokinase deficiency results essentially from the defective regulation of Kₐᵥ channels, rather than from nonelectrical consequences of altered metabolism. Further indirect support for this hypothesis comes from: (1) the demonstration that mice with ATP-insensitive β-cell Kₐᵥ channels also die from neonatal diabetes (19) and (2) the recent dramatic demonstration that gain-of-function Kₐᵥ mutations also cause permanent neonatal diabetes in humans (20–24). A critical prediction is that the conse-
disruption of the pore-forming Kir6.2 subunit (25). KATP euglycemia as adults (25–28). By crossing nGK with transient hyperinsulinemia and hypoglycemia as necquences of nGK deficiency should be abrogated in animals to Kir6.2 knockout mice (Kir6.2<sup>−/−</sup> mice, we can test influerences on glucose metabolism is lost, and both pathways (1 and 2) will be “off.” In nGK<sup>−/−</sup> mice, the Kir6.2-dependent pathway (1) will be permanently “on,” and glucose-dependent regulation will then PKC, protein kinase C; VDCC, voltage-dependent Ca<sup>2+</sup> channel.

RESULTS

Neonatal survival of nGK knockout mice on the Kir6.2 knockout background. Of 57 live births from cross-breeding of heterozygous nGK<sup>−/+</sup> mice on the Kir6.2 wild-type background, only 2 mice were positively genotyped as nGK<sup>−/−</sup> (Fig. 2A). Both were found dead within the 1st week (genotyped postmortem) after birth (Fig. 2B). Conversely, on the Kir6.2 knockout background, nGK<sup>−/−</sup> pups were born at the expected ratio (1:2:1 for nGK<sup>−/−</sup>/Kir6.2<sup>−/−</sup>, nGK<sup>−/−</sup>/Kir6.2<sup>+/−</sup>, and nGK<sup>−/−</sup>/Kir6.2<sup>+/+</sup>, respectively) (Fig. 2A). These double-knockout mice survived much longer than nGK<sup>−/−</sup> on the Kir6.2 wild-type background, with a median survival time of ~10 days, and ~30% surviving past weaning (21 days) (Fig. 2B). As is shown in Fig. 2C, double-knockout nGK<sup>−/−</sup>/Kir6.2<sup>−/−</sup> mice were significantly smaller than both nGK<sup>−/−</sup>/Kir6.2<sup>+/−</sup> and nGK<sup>−/−</sup>/Kir6.2<sup>+/+</sup> mice at 4 weeks of age. By 5 weeks of age, nGK<sup>−/−</sup>/Kir6.2<sup>−/−</sup> mice weighed ~50% (4.78 ± 0.17g) that of nGK<sup>−/−</sup>/Kir6.2<sup>−/−</sup> (8.34 ± 0.24g) or nGK<sup>−/−</sup>/Kir6.2<sup>−/−</sup> (9.02 ± 0.17g.) littermates. Nevertheless, it is striking that these double-knockout mice, which completely lack β-cell glucokinase activity, can survive beyond weaning.

On this Kir6.2<sup>−/−</sup> background, fasting blood glucose was only slightly higher in nGK<sup>−/−</sup> than in nGK<sup>−/−</sup> or nGK<sup>−/−</sup> mice (Fig. 3A). However, fed glucose (Fig. 3B) was dramatically higher in nGK<sup>−/−</sup> mice, suggesting that these mice either still do not secrete enough insulin to lower blood glucose values or that they develop insulin resistance (see below).

For control of effects of genetic background, comparisons between genotypes were made with littermates in every experiment, and n values of at least three are reported in each paired genotype. Thus, it is reasonable to conclude that any effects of nonmutated chromosomes will be randomized within all genotypes.
nGK<sup>−/−</sup> animals are diabetic on the Kir6.2<sup>+/+</sup> background but not on the Kir6.2<sup>−/−</sup> background. Heterozygous nGK<sup>+/−</sup> mice were born at close to expected ratios on both the Kir6.2<sup>+/+</sup> and Kir6.2<sup>−/−</sup> backgrounds (Fig. 2A) and developed apparently normally. As previously reported, on the wild-type Kir6.2<sup>+/+</sup> background, heterozygous nGK<sup>+/−</sup> mice show nonprogressive mild diabetes, as in human MODY-2. At birth, the blood glucose levels of nGK<sup>+/−</sup> mice were similar (3.5 ± 0.4 and 3.9 ± 0.2 mmol/l, respectively), but by 12 weeks, nGK<sup>+/−</sup> mice showed a twofold increase in both fasting and fed blood glucose compared with nGK<sup>+/−</sup> mice (Fig. 3A and B).

By contrast, on the Kir6.2 knockout background, overt diabetes, as evidenced by elevated fasting glucose levels, is ameliorated in nGK<sup>+/−</sup> mice, although fed glucose levels are elevated (Fig. 3A and B). Plasma insulin measured under fed conditions was slightly elevated in nGK<sup>+/−</sup> mice versus nGK<sup>−/−</sup> on both Kir6.2<sup>+/+</sup> and Kir6.2<sup>−/−</sup> backgrounds (Fig. 3C). Enhance enhancement in glucose tolerance of heterozygous nGK-deficient mice in the absence of K<sub>ATP</sub> channel.
Kir6.2 knockout abrogates nGK−/− mice (Fig. 4), to the extent that they were not significantly different from nGK+/− mice on the same background. These data thus reiterate the perplexing result that Kir6.2−/− mice on the Kir6.2 background, and Kir6.2−/− mice were not different between the various genotypes (for example, islet diameter was 175.5 ± 8.1 μm for nGK+/−Kir6.2+/− and 183.3 ± 7.3 μm for nGK+/−Kir6.2−/−, n = 35 islets). Glucose-stimulated insulin secretion (GSIS) was assessed in isolated islets from all five available genotypes. As shown previously, the response of nGK−/− islets was markedly reduced compared with nGK+/−, on the Kir6.2+/+ background (15). On the Kir6.2−/− background, GSIS was greatly reduced in the presence or absence of nGK (Fig. 6A), and there was no enhancement of release from nGK+/− or nGK−/−, relative to the release from the same nGK genotypes on the Kir6.2+/− background. These data thus rule out the perplexing result that Kir6.2−/− (25) and SURI−/−(26) mice are mildly glucose tolerant, yet have only minimal GSIS. Moreover, these results do not help to explain the enhanced glucose tolerance and survival of nGK-deficient mice on this Kir6.2−/− background. Recent studies have begun to shed light on the potential mechanisms in SURI−/− mice (30–32). In particular, glutamine has been shown to preferentially stimulate KATP-independent secretion from SURI−/− islets (31). This effect has not been examined in Kir6.2−/− islets, but if the phenotypes of SURI−/− and Kir6.2−/− indeed both result from loss of KATP, we would expect the same KATP-independent stimulation of Kir6.2−/− islets. As shown in Fig. 6B, glutamine markedly stimulates secretion from both nGK+/− and nGK+/− islets on the Kir6.2−/− background relative to the wild-type background. Given likely physiological levels of glutamine, this may well cause enhanced insulin secretion in vivo and explain the improved glucose tolerance of nGK−/− on the Kir6.2−/− background (31) (see below).

β-Cell-specific reduction of KATP channel activity enhances glucose tolerance of nGK−/− mice. Although the above data are consistent with a specific loss of KATP channels in β-cells being responsible for abrogation of the nGK-deficiency phenotype, it is a caveat that the Kir6.2 knockout is global. We previously generated β-cell-specific dominant-negative Kir6.2[AA] mice using an insulin promoter to drive transgene expression (29). Kir6.2[AA] mice, which lack KATP in ~70% of β-cells, hypersecrete insulin and have slightly enhanced glucose tolerance (29). In the current study, we bred these mice with nGK−/−Kir6.2−/− mice to generate double-heterozygous (nGK+/−Kir6.2[AA]) mice (see Research Design and Methods). As shown in Fig. 7, these mice have a marked improvement in glucose tolerance compared to nGK+/− alone. Although the improvement is not as dramatic as in the mice completely lacking KATP (Fig. 4), this result is consistent with β-cell KATP channels indeed being the major players in rescuing the nGK-deficiency phenotype.
similarly suppressed in nGK secretion during hyperglycemia (2,15). Rates, but they have impaired glucose tolerance and insulin mild hyperglycemia with normal basal glucose turnover type, mice lacking one allele of neuroendocrine GK exhibit severe diabetes (15,33). Consistent with a MODY-2 pheno-

typical lethality is clearly abrogated (Fig. 2), weight, severe diabetes, and died prematurely, but perinatal

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heterozygous nGK+/− mice. On the Kir6.2+/− background, they have markedly elevated fed and fasting glucose levels, and they are markedly glucose intolerant. By contrast, on the Kir6.2−/− background, nGK+/− mice have near-normal fasting glucose levels and similar glucose tolerance to nGK+/−Kir6.2−/− animals. The abolition of KATP significantly ameliorates the “MODY-2” phenotype of the nGK+/− mice.

**Complex phenotypes of Kir6.2-deficient × nGK-deficient mice.** The initial prediction that, by removing the electrical defect, the diabetic consequences of nGK deficiency should be significantly avoided on the Kir6.2−/− background was thus supported. However, the mechanistic consequences of the genetic alterations are not absolutely clear. The naive expectation of mice lacking KATP channels was a persistent hyperinsulinemic phenotype because of unregulated secretion, but several studies have now shown that this expectation is not met (25–27). For unexplained reasons (but see below), Kir6.2−/− mice are normoglycemic, with slightly impaired glucose tolerance and reduced GSIS. Similarly, although glucose tolerance was greatly improved in nGK+/− mice on the Kir6.2−/− background, GSIS in isolated islets was still notably reduced (Fig. 6A). However, as has recently been shown, the sensitizing effect of amino acids, in particular glutamine (30,31), is much greater in islets that lack KATP (SUR1−/−). Similarly, we find that Kir6.2−/− islets can show enhanced insulin secretion at physiological glucose concentration (compared with Kir6.2+/− mice) in the presence of stimulatory glutamine (Fig. 6F). This enhancement is seen in both nGK+/− and nGK+/− islets on this background. We suggest that, in vivo, ambient glutamine levels may lead to such an enhancement of insulin secretion and that, in part, this underlies the abrogating effect of KATP deficiency on nGK-deficiency (31).

Because we are examining a global neuroendocrine glucokinase deficiency, it is conceivable that the effects of Kir6.2 knockout are mediated through extra-pancreatic consequences, although insulin tolerance tests (Fig. 5) show that nGK−/−Kir6.2−/− mice are no more insulin sensitive than nGK+/−Kir6.2−/− mice, excluding enhanced insulin sensitivity as an explanation for the rescued phenotype. Further evidence for a pancreatic basis for the Kir6.2-deficient rescue of nGK deficiency would be a β-cell–specific KATP knockout. A tissue-targeted knockout has not been generated, although we have generated an insulin promoter–driven dominant-negative Kir6.2[AAA] mouse (29) that lacks KATP in ~70% of its β-cells (29). The demonstration of marked improvement in glucose tolerance in double-heterozygous (nGK+/−Kir6.2[AAA]) mice, compared with nGK+/− alone (Fig. 7), provides further support for β-cell–specific KATP channels indeed being the major players in rescuing the nGK-deficiency phenotype.

**Relevance to glucokinase-deficiency diabetes in humans.** The significant reduction in fasting glucose, slight yet significant decrease in fed glucose concentration, and marked enhancement in glucose tolerance in nGK+/−Kir6.2−/− mice with respect to nGK+/−Kir6.2+/− mice suggests that, at least in part, the MODY-2 phenotype is abrogated on the KATP knockout background, highlighting the importance of electrical signaling in the regulation of insulin secretion. Decreased insulin secretion in response to glucose from nGK−/−Kir6.2−/− or nGK+/−Kir6.2−/− islets recapitulates the response observed in nGK+/−Kir6.2−/− islets, thus indicating that the impaired GSIS is due to the Kir6.2 knockout phenotype and not due to glucokinase deficiency. Most patients with heterozygous GK mutations do not require pharmacological treatment, and the majority of cases are managed with diet alone. However, during pregnancy, women with GK mutations are often treated with insulin to maintain normal blood sugar levels, resulting in babies that are large for gestational age because of the effect of insulin on fetal growth (35). In these cases, sulfonylurea treatment could presumably be a valid alternative treatment, given the normalizing effect of KATP deficiency that we now demonstrate, because nGK−/−Kir6.2+/− transgenic mice do respond to glibenclamide treatment in vitro (15).

Taken together, the results from nGK-deficient and KATP-deficient crosses show a marked improvement in survivability and glucose tolerance of the former on the KATP null background. Neonatal lethality of nGK−/−Kir6.2+/− mice is avoided on the Kir6.2−/− background, demonstrating that this consequence is attributable to loss of metabolic coupling between glucose and insulin secretion, probably through electrical signaling. As with the now dramatically improved treatment options for KATP−/− induced permanent neonatal diabetes (21,24), we suggest that there may be a role for sulfonylurea therapy in permanent neonatal diabetes resulting from glucokinase deficiency (13). A significant number of nGK−/−Kir6.2+/− mice survived beyond weaning. This should also allow more extensive studies of the nonelectrical consequences (which are still ultimately lethal) of total glucokinase deficiency. Such studies will have the potential to inform and further improve treatment options for diabetes resulting from glucokinase deficiency.

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