Original Article

Expression of ATP-Insensitive K_{ATP} Channels in Pancreatic β-Cells Underlies a Spectrum of Diabetic Phenotypes

Joseph C. Koster, Maria S. Remedi, Ricard Masia, Brian Patton, Ailing Tong, and Colin G. Nichols

Glucose metabolism in pancreatic β-cells elevates cytoplasmic [ATP]/[ADP], causing closure of ATP-sensitive K^+ channels (K_{ATP} channels), Ca^{2+} entry through voltage-dependent Ca^{2+} channels, and insulin release. Decreased responsiveness of K_{ATP} channels to the [ATP]/[ADP] ratio should lead to decreased insulin secretion and diabetes. We generated mice expressing K_{ATP} channels with reduced ATP sensitivity in their β-cells. Previously, we described a severe diabetes, with nearly complete neonatal lethality, in four lines (A–C and E) of these mice. We have now analyzed an additional three lines (D, F, and G) in which the transgene is expressed at relatively low levels. These animals survive past weaning but are glucose intolerant and can develop severe diabetes. Despite normal islet morphology and insulin content, islets from glucose-intolerant animals exhibit reduced glucose-stimulated insulin secretion. The data demonstrate that a range of phenotypes can be expected for a reduction in ATP sensitivity of β-cell K_{ATP} channels and provide models for the corollary neonatal diabetes in humans. *Diabetes* 55:2957–2964, 2006

In the pancreatic β-cell, changes in electrical activity tightly control the release of insulin necessary to maintain blood glucose levels within a narrowly defined physiologic range. Perturbations in electrical activity are predicted to impair insulin release and result in inappropriate serum insulin levels for a given blood glucose concentration. A persistent hyperexcitabilty of the β-cell results in unregulated insulin release and underlies hyperinsulinism with hypoglycemia in humans (1). Conversely, it is now clear that hypoexcitability can suppress insulin release and contribute to the converse disorder of diabetes (2).

The ATP-sensitive K^+ channel (K_{ATP} channel) is the critical link between cell metabolism and electrical activity of the β-cell. Under conditions of low glucose, K_{ATP} channels provide the dominant β-cell membrane conductance, maintaining the cell in a hyperpolarized state. Conversely, in the fed state, glucose entering the β-cell is rapidly metabolized, causing a rise in the cytoplasmic [ATP]/[ADP] ratio, ATP-driven closure of K_{ATP} channels, membrane depolarization, and voltage-dependent Ca^{2+} entry. Calcium influx, in turn, triggers insulin release.

K_{ATP} mutations that result in loss of ATP sensitivity should decrease membrane excitability and impair insulin secretion. A clear picture is now emerging from both animal and human studies that such K_{ATP} mutations can cause hyposecretion and underlie diabetes (2). Genetic studies have now identified mutations in the pore-forming Kir6.2 subunit of K_{ATP} (*KCNJ11*) as a common cause of neonatal diabetes mellitus (NDM) in humans (3–9). NDM is a rare but severe diabetes that is diagnosed within the first few weeks of life and has typically required life-long insulin injections to treat (10). In the most severe cases, NDM is part of a neurological syndrome that includes developmental delay, epilepsy, and neonatal diabetes (3,11). All Kir6.2 mutations that have been functionally characterized result in a reduction of ATP sensitivity and are predicted to cause an increase in K_{ATP} activity in the β-cell (3,7,8,12–15). Previously, we described a severe neonatal diabetic phenotype in transgenic mice expressing K_{ATP} channels with similarly reduced ATP sensitivity (i.e., "gain-of-function" K_{ATP} channels) in their pancreatic β-cells (16). All transgenic offspring of four founder lines (A–C and E) expressing the Kir6.2[ΔN30] transgene exhibited hypoinsulinemia with ketoacidosis and usually died within the 1st week of life. Islet morphology and insulin localization was normal at the earliest stages of the disease, implicating reduced insulin secretion as causal.

K_{ATP}-induced neonatal diabetes in humans may be permanent or transient in form (3,7). In addition, a Kir6.2 mutation (C42R) has recently been linked to neonatal diabetes, gestational diabetes, and later-onset ostensibly type 1 or type 2 diabetes within a single pedigree (8). These findings raise the intriguing possibility that Kir6.2 mutations may contribute to a spectrum of disease phenotypes, including later developing and presumably milder forms of the disease. Consistent with this notion, numerous control-based genetic studies have identified a Kir6.2 polymorphism (E23K) as a risk factor in the development of type 2 diabetes (17–20). A reduced insulin secretory capacity in nondiabetic carriers of the E23K polymorphism implicates β-cell dysfunction (19,21). Variable clinical presentations have also been reported in other monogenic forms of diabetes, e.g., mutations in the genes encoding hepatocyte nuclear factor-1α and -1β (22).

From the Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, Missouri.

Address correspondence and reprint requests to C.G. Nichols, Department of Cell Biology and Physiology, Washington University School of Medicine, 660 South Euclid Ave., St. Louis, MO 63110. E-mail: cnichols@cellbiology.wustl.edu.

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GFP, green fluorescence protein; GSIS, glucose-stimulated insulin secretion; GTT, glucose tolerance test; HFD, high-fat diet; K_{ATP} channel, ATP-sensitive K^+ channel; NDM, neonatal diabetes mellitus; RIP I, rat insulin promoter I.

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To date, the only animal model of K_{ATP} "gain-of-function" mutations is that reported in our previous study (16), which reiterates severe NDM. We have now examined another three lines (D, F, and G) of mice expressing the Kir6.2[ΔN2-30] transgene in some but not all β-cells. In these mice, severe NDM and nearly complete neonatal lethality is absent. However, these transgenic mice display a spectrum of diabetic phenotypes ranging from euglycemia with impaired glucose tolerance as adults to a severe overt diabetes developing around weaning. Isolated islets from glucose-intolerant, adult transgenic mice express pancreatic K_{ATP} with reduced ATP sensitivity and exhibit a reduction in glucose-stimulated insulin secretion (GSIS), despite normal islet morphology and insulin content. In the severe diabetic mouse, there is further reduction in GSIS and evidence for disruption of islet morphology, implying a secondary progression of the diabetes as a consequence of K_{ATP} overactivity. These animals provide unique models for the graded levels of K_{ATP} overactivity and the varying consequences for glucose tolerance and diabetes.

RESEARCH DESIGN AND METHODS

Construction of mutant Kir6.2 construct. Generation of the Kir6.2[ΔN2-30] transgene was described previously (16). The Kir6.2[ΔN2-30] subunit construct containing a green fluorescence protein (GFP) tag, was excised as an *Eco*RI/*Eco*RI fragment from the pCMV6b parent plasmid and inserted downstream of the rat insulin promoter I (RIP I) in the transgenic plasmid pRIP I (23). The entire transgenic construct was excised from Kir6.2[ΔN2-30]–pRIP I plasmid as a *Sac*I/*Xho*I fragment for microinjection.

Generation of transgenic mice. The Kir6.2[ΔN2-30] DNA construct was microinjected into fertilized eggs of C57Bl6 X CBA mice according to standard technique (24) at the Washington University in St. Louis Mouse Genetics Core Facility (http://mgc.wustl.edu/). Seven transgenic founder mice (A–G) were identified and, in the present studies, mice from the D, F, and G founder lines were used. Transgenic mice were identified by PCR on mouse-tail DNA using GFP-specific oligonucleotide primers.

Isolation of pancreatic islets and β-cells. Mice were killed by cervical dislocation. Pancreata were removed and injected with Hank's solution containing collagenase (in mmol/l: 137 NaCl, 5.4 KCl, 4.2 $NaHCO_3$, 1.3 $CaCl_2$, 0.44 KH_2PO_4, 0.4 $MgSO_4$, 0.3 $NaHPO_4$, 1 EGTA, 5.6 D-glucose, and collagenase [0.5 mg/ml], pH 7.4). Collagenase type XI was obtained from Sigma-Aldrich (St. Louis, MO). Pancreata were digested for 5 min at 37°C and washed three times in cold Hank's solution. Islets were isolated by hand under a dissecting microscope, and pooled islets were maintained in CMRL-1066 culture medium (Gibco) supplemented with FCS (10%), penicillin (100 units/ml), and streptomycin (100 μg/ml). For electrophysiologic recordings, islets were incubated for 20 min at 37°C in Ca^{2+}-free glucose solution (in mmol/l: 138 NaCl, 5.6 KCl, 1.2 $MgCl_2$, 1 EGTA, 10 HEPES, 3 D-(+)-glucose, pH 7.35) and then mechanically agitated in order to disperse islets into individual β-cells. Dispersed β-cells were plated on glass coverslips and allowed to attach for 1 h at 37°C. Cells were maintained up to 3 days at 37°C in CMRL-1066 medium in a humidified incubator. Confocal imaging of isolated islets was performed using a Zeiss laser scanning confocal microscope.

Immunohistological analysis. Pancreata were fixed in 10% formalin and paraffin embedded for serial sectioning (5-μm thick). For immunofluorescence, pancreatic sections were incubated overnight at 37°C with a guinea pig anti-insulin primary antibody (1:250; Linco Research, St. Charles, MO) or a guinea pig anti-glucagon primary antibody (1:500; Linco Research). Primary antibodies were detected by incubating for 1.5 h at 25°C with an anti–guinea pig secondary antibody conjugated with the Alexa 488 fluorescent dye (Molecular Probes, Eugene, OR).

Electrophysiologic measurements. Patch-clamp experiments were performed as previously described (25). Briefly, the standard bath (intracellular) and pipette (extracellular) solutions used in these experiments (K_{int}) had the following composition: 140 mmol/l KCl, 10 mmol/l K-HEPES, 1 mmol/l K-EGTA, pH 7.3. All currents were measured at a membrane potential of −50 mV (pipette voltage = +50 mV). Inward currents at this voltage are shown as upward deflections. Data were normally filtered at 0.5–3 kHz, and signals were digitized at 22 kHz (Neurocorder; Neurodata, New York, NY). Experiments were digitized into a microcomputer using Clampex 8.2 software (Axon), and off-line analysis was performed using Clampfit 8.2 (Axon) and Microsoft Excel programs. Wherever possible, data are presented as means ± SE. Microsoft Solver was used to fit data by a least-square algorithm.

Measurements of blood glucose and serum insulin levels. Blood glucose concentration was assayed using the Bayer Glucometer Elite XL glucose meter (Elkhart, IN). The upper limit of glucose detection is 600 mg/dl and the lower limit 20 mg/dl. All glucose values that were above the limit of detection are presented as >600 mg/dl. Blood insulin levels were assayed on 5 μl mouse serum using the Rat Insulin ELISA Kit with a mouse insulin standard (Crystal Chemical, Chicago, IL).

Insulin release experiments. Following overnight incubation in CMRL medium, pancreatic islets (five per well in a 12-well plate) were preincubated in glucose-free Dulbecco's modified Eagle's medium, supplemented with 3 mmol/l D-(+)-glucose for 1.5 h. The release assay was initiated by supplementing the Dulbecco's modified Eagle's medium glucose-free media with D-(+)-glucose (1, 7, 16.7, or 23 mmol/l) as indicated. Islets were incubated for 60 min at 37°C and medium removed and assayed for insulin content using Rat Insulin RIA (Linco). Each experiment was repeated in triplicate.

RESULTS

Expression of an ATP-insensitive Kir6.2 transgene in pancreatic β-cells. Truncation of the NH_2-terminal 30 amino acids of the Kir6.2 subunit results in an ~10-fold decrease in ATP sensitivity of K_{ATP} channels when reconstituted with SUR1 (25). This change in ATP sensitivity is similar in magnitude to changes observed with Kir6.2 mutations associated with NDM in humans (2). To investigate the effect of mutant K_{ATP} channels with altered ATP sensitivity on insulin release in pancreatic β-cells, the NH_2-terminal truncated subunit, Kir6.2[ΔN2-30], was fused at the COOH-terminus with the GFP and cloned downstream of the RIP I in order to drive expression specifically in pancreatic β-cells (23). From >400 injected embryos, PCR analysis identified seven founder mice that were bred with C57Bl/6 mice to establish seven founder lines (lines A–G). We have previously described the phenotype of four founder lines (A–C and E), each of which exhibited a severe NDM that was correlated with transgene expression (16). These mice show a very similar diabetic phenotype to the untreated human permanent NDM, including profound hyperglycemia, hypoinsulinemia, ketoacidosis, and small size (3). For the current study, we have examined the phenotype of three additional (D, F, and G) lines that consistently exhibit positive expression for the Kir6.2[ΔN2-30] transgene but do not show the severe neonatal phenotype.

Each of the founder D, F, and G mice was euglycemic, developed normally, and was fertile. In sharp contrast to F1 mice from the A–C and E line mice (16), neonatal lethality was not typically observed in F1 (or above) offspring of D, F, or G line founder mice. Approximately 81% of G line transgenic mice (76 of 94) and 84% of F line transgenic mice (81 of 96) survived to weaning (day 21). D line transgenic mouse survival was not monitored in detail, but was >90% for >25 births. Consistent with expression of the Kir6.2[ΔN2-30] transgene, green fluorescence was visible in islet cells from Kir6.2[ΔN2-30] transgenic mice but not in islets from control mice (Fig. 1). Typically, 30–50% of the islet cells from F and G line Kir6.2[ΔN2-30] transgenic mice expressed visible levels of the GFP transgene, although GFP expression was highly variable with some transgenic islets exhibiting considerably fewer fluorescing β-cells (Fig. 1*A*, *lower panel*). Functional and genetic mosaicism is common in transgenic mice and is likely to underlie this phenotypic variability (26,27).

Impaired glucose tolerance in adult transgenic Kir6.2[ΔN2-30] mice. The whole-animal phenotype of D, F, and G line transgenic mice initially appeared benign.

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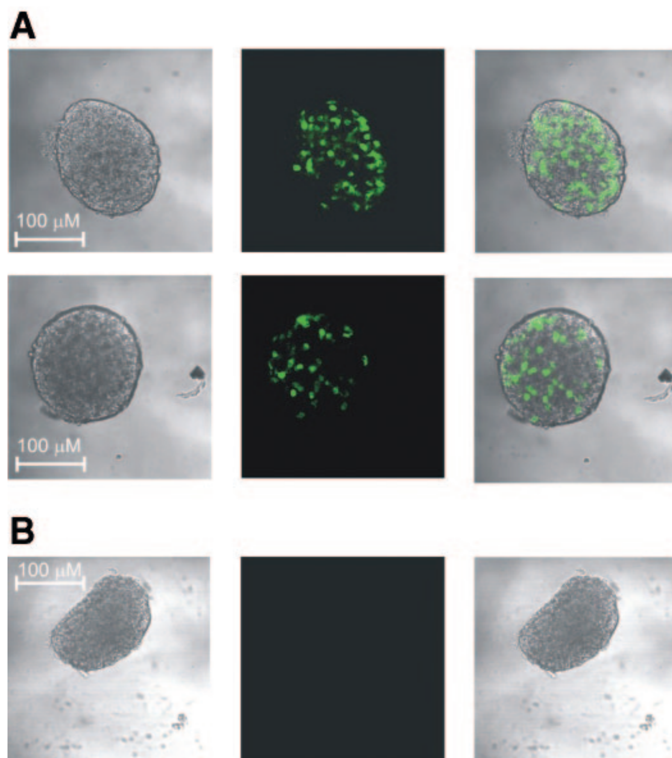

FIG. 1. Fluorescence of GFP-tagged K_{ATP} channels in islets from transgenic mice. Representative clear field and fluorescent confocal images of isolated islets from an adult Kir6.2[ΔN2-30] transgene mouse (*A*) and a control littermate mouse (*B*). Kir6.2[ΔN2-30] transgene mouse: age 11 months, random blood glucose 170 mg/dl; control mouse: age 11 months, random blood glucose 127 mg/dl.

However, random measurements of blood glucose in fed mice occasionally indicated abnormally high levels. Figure 2*A* shows the results of a "case study" of one litter of F line mice, in which afternoon blood glucose levels were measured (without respect to feeding status), over the course of ~1 year. Significant variability was apparent in transgenic mice but not in nontransgenic littermates. After almost a year, no mouse in the litter was overtly diabetic and there was no significant difference in either body weight or fasting blood glucose measurements in transgenic mice compared with control littermates (data not shown). To examine the possibility that glucose handling was impaired, and underlying the variability of blood glucose levels, controlled glucose tolerance tests (GTTs) were performed. As shown in Fig. 2*B*, transgenic mice indeed exhibited significantly lower glucose tolerance than controls.

A high-fat diet (HFD) exacerbates a hyperglycemic phenotype by decreasing peripheral insulin sensitivity and taxing the β-cell to respond with increased insulin secretion (28). However, the effects of an HFD on a primarily insulin secretory–deficient phenotype is not known. As shown in Fig. 2*C*, Kir6.2[ΔN2-30] transgenic and littermate control mice on an HFD for 3 months were each significantly more glucose intolerant than their respective controls on a normal diet. Importantly, the difference in glucose tolerance between the control and Kir6.2[ΔN2-30] mice was further increased on an HFD, even though there was no significant difference in the body weight increases (Fig. 2). As shown in Fig. 3*A*, there is a trend toward lower serum insulin levels and higher blood glucose values in adult transgenic mice on both a normal diet and an HFD as compared with controls. This difference was confirmed during a GTT in which insulin levels were lower and glucose values higher in transgenic mice at 30 min post-glucose injection (Fig. 3*B*).

Cellular basis of the insulin secretory deficit. Insulin secretion was assayed in vitro from pancreatic islets isolated from nondiabetic, adult transgenic mice on a normal diet. There was no appreciable difference in insulin content between transgenic and control islets (Fig. 4*B*). Basal insulin secretion was also not different, but at higher glucose concentrations (16 and 23 mmol/l), a suppression of insulin release was observed with the transgenic islets (Fig. 4*A*). A similar reduction in insulin release was observed at a stimulatory glucose concentration in islets derived from mice on an HFD (insulin release [ng/10 islets/h] in 16 mmol/l glucose = 92 ± 29 for control islets [n = 9 mice] and 29 ± 7 for Kir6.2[ΔN2-30] islets [n = 8 mice]).

To examine the cellular basis of the diabetic phenotype, K_{ATP} currents were assessed in pancreatic β-cells. As shown in Fig. 4*C* and *D*, K_{ATP} channels in transgenic β-cells isolated from nondiabetic adult mice displayed a significant (threefold) decrease in ATP sensitivity relative to β-cell K_{ATP} channels from control littermates. Optical limitations of the recording system allow for visual detection of only the highest fluorescing β-cells, which, presumably, express the most ATP-insensitive Kir6.2[ΔN2-30]–containing channels. In the subpopulation of transgenic β-cells with visibly detectable GFP fluorescence, the reduction in ATP sensitivity was even greater (approximate eightfold decrease).

Secondary development of severe diabetes postweaning. Although neonatal lethality was not present in transgenic F or G line mice, a severe and persistent diabetes developed in a minority of these mice around the time of weaning (6 of ~86 transgenic births, i.e., 7%; ages ranging from 30 to 60 days at the time diabetes was observed). The severely diabetic mice generally showed a pronounced lethargy and smaller size than littermates. At the time of diagnosis, these animals exhibited ~20% lower body weight than nontransgenic littermates (body weight 24 ± 1.6 g [littermate n = 5] and 19.4 ± 1.6 g [transgenic n = 5]; $P \leq 0.01$, Student's paired t test). In each case, multiple blood glucose measurements (three or more over 2 days) were >600 mg/dl, even though normal blood glucose levels (100–200 mg/dl) had been recorded at earlier ages in some cases.

When severely diabetic mice were detected, mice were generally killed within 48 h and the islets isolated. In contrast to the reduced GSIS observed in islets from the glucose-intolerant animals, these animals showed a virtually complete loss of GSIS, together with an apparent reduction of insulin content (Fig. 5). These mice demonstrate that a secondary progression, from glucose intolerance to diabetes, can occur and that such progression is associated with significant deterioration of islet function.

Islet morphology in transgenic Kir6.2[ΔN2-30] mice. Immunohistochemistry was performed on pancreatic sections from glucose-intolerant adult mice (Fig. 6*A*) and from spontaneously diabetic mice (Fig. 6*B*), as well as age-matched nontransgenic littermates. As shown in Fig. 6*A*, islet morphology was preserved in glucose-intolerant islets, with strong staining of insulin-containing β-cells in the core of the islet and peripheral staining of glucagon-containing α-cells. In contrast, in islets from a spontaneously diabetic Kir6.2[ΔN2-30] mouse, disruption of the

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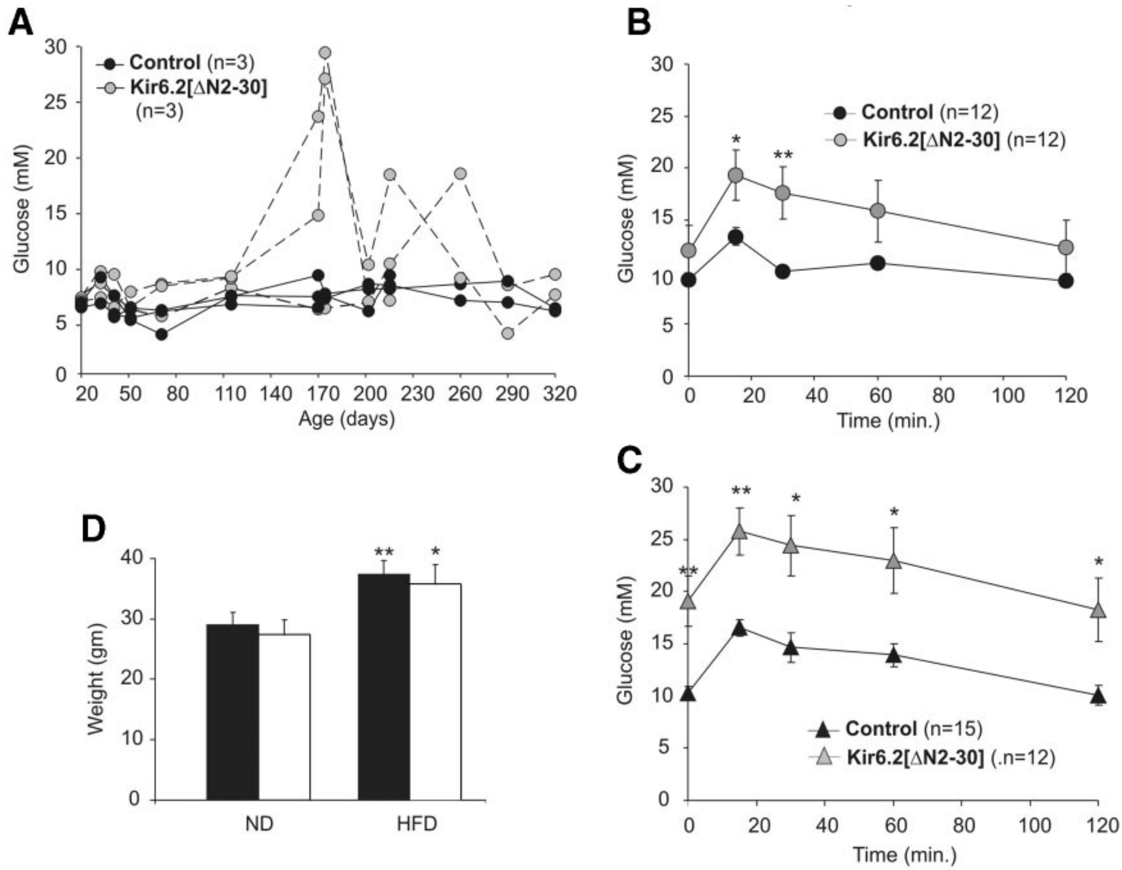

FIG. 2. Blood glucose homeostasis in adult Kir6.2[ΔN2-30] transgenic and control littermate mice. *A*: Random blood glucose measurements in a single litter of transgenic ($n = 3$) and control mice ($n = 3$) from age 21 to 321 days. One of the transgenic mice died during the experiment of an unknown cause at day 220. *B*: Blood glucose (mg/dl) versus time following intraperitoneal glucose injections (1.5 g/kg body wt) after overnight fasting (16 h). Intraperitoneal GTT results from Kir6.2[ΔN2-30] transgenic mice ($n = 12$, ages 7–18 months) and control littermate mice ($n = 12$, ages 7–18 months) on a normal diet. *C*: Intraperitoneal GTT results from transgenic mice ($n = 12$, ages 6–18 months) and control littermate mice ($n = 15$, ages 6–18 months) after 3 months on an HFD. *D*: Body weights from Kir6.2[ΔN2-30] transgenic (□) and control littermate (■) mice on a normal diet (ND) or after 3 months on an HFD. Data represent means ± SE. *$P < 0.05$, **$P < 0.01$, unpaired Student's t test.

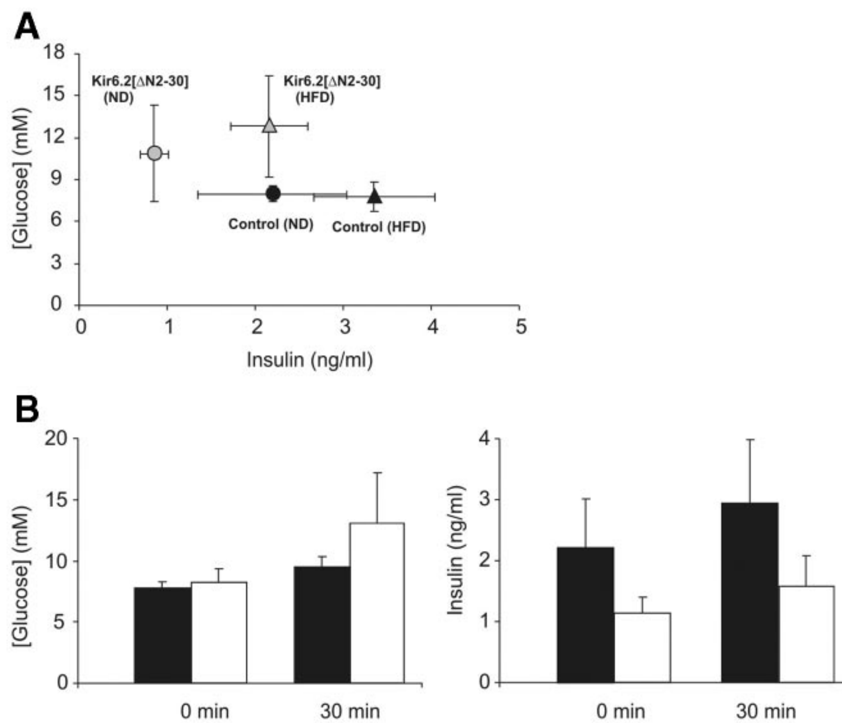

FIG. 3. Blood glucose and serum insulin levels from glucose-intolerant adult Kir6.2[ΔN2-30] mice and control littermates on a normal diet or an HFD. *A*: Blood glucose versus serum insulin in random-fed control or transgenic mice. For control mice on normal diet (ND): $n = 9$, aged 6–23 months; transgenic mice on normal diet: $n = 8$, aged 6–23 months. For control mice on an HFD: $n = 5$, aged 7–21 months; transgenic mice on an HFD: $n = 8$, aged 7–21 months. *B*: Blood [glucose] (*left*) and plasma insulin (*right*) before and 30 min after intraperitoneal glucose loading (1.5 g/kg body wt). Control mice (HFD): $n = 5$, aged 9–21 months; transgenic mice (HFD): $n = 5$, aged 9–21 months. Data represent means ± SE. ■, control; □, Kir6.2[ΔN2-30].

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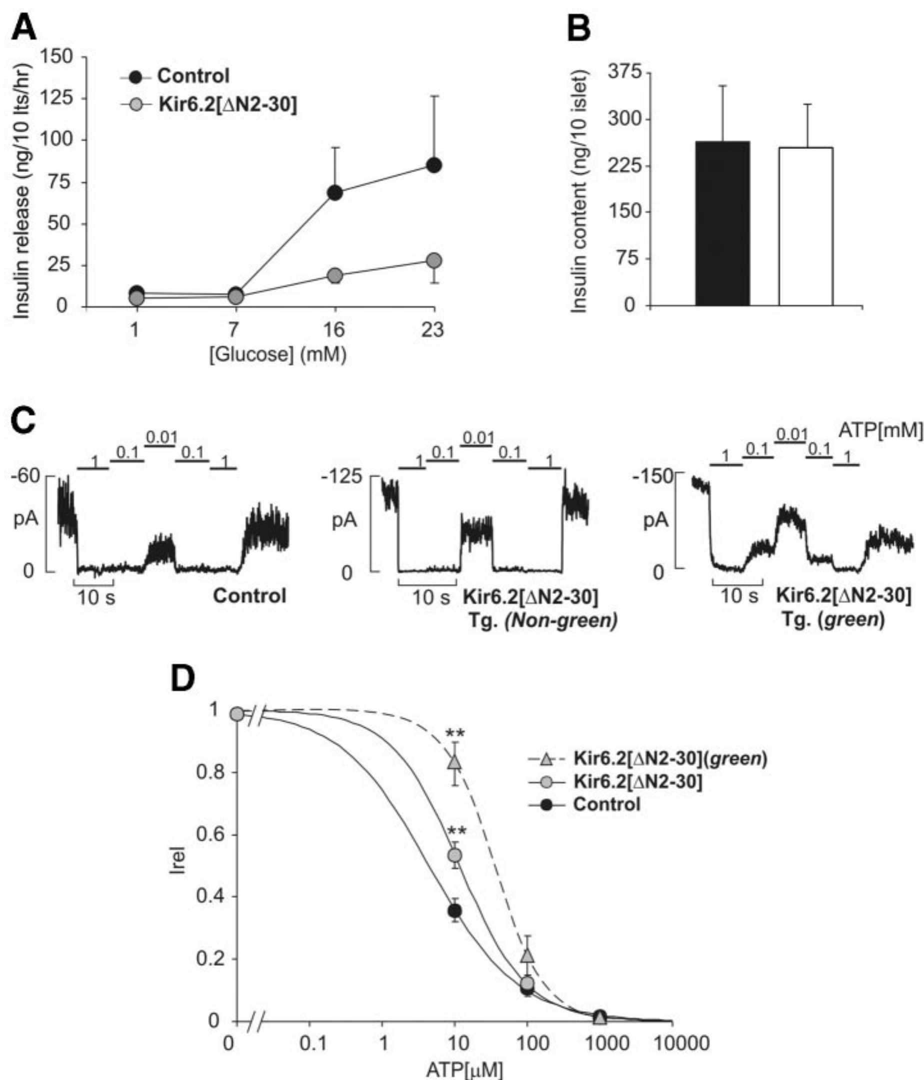

FIG. 4. Cellular basis of insulin secretory failure in adult, glucose-intolerant Kir6.2 [ΔN2-30] mice. *A*: Insulin release was assayed from isolated islets (five islets per well) in triplicate following a 1-h incubation in medium containing varying [glucose] as shown. Control: *n* = 5–9 mice, aged 4–22 months; transgenic: *n* = 5–9 mice, aged 4–22 months. Fed blood glucose = 123.4 ± 5.0 mg/dl (control) and 129.6 ± 6.6 mg/dl (transgenic). All mice were fed a normal diet. *B*: Total insulin content from nondiabetic adult control littermate (*n* = 8) and transgenic mice (*n* = 8). ■, control; □, Kir6.2[ΔN2-30]. *C*: Representative K_{ATP} currents recorded from inside-out membrane patches from pancreatic β-cells isolated from adult, nondiabetic Kir6.2[ΔN2-30] transgenic mice or control littermates. K_{ATP} currents were measured at −50 mV in K_{int} solution and patches were exposed to varying [ATP] as shown. Current trace on the *right* is from a fluorescing β-cell expressing the GFP-tagged transgene. *D*: Steady-state dependence of membrane current on [ATP] (relative to current in zero ATP [I_{rel}]) from control (*n* = 11 patches from three mice) and transgenic (*n* = all 26 patches from four mice and four green patches from two mice) membrane patches. Data points represent the means ± SE in each case. Fitted lines correspond to least-squares fits of the Hill equation: relative current = $100/(1 + [ATP]/K_i)^H$, with H (Hill coefficient) free to vary. Mean values were K_i = 4.4 μmol/l (control), 11.7 μmol/l (Kir6.2[ΔN2-30] transgenic), and 33.4 μmol/l (Kir6.2[ΔN2–30] transgenic [green]), and H = 0.7 (control), 1.0 (Kir6.2[ΔN2-30] transgenic), and 1.3 for (Kir6.2[ΔN2-30] transgenic [green]). ***P* < 0.01, unpaired Student's *t* test.

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islet morphology was evidenced by an infiltration of glucagon-containing α-cells into the core of the islet (Fig. 6*B*). The disruption of islet morphology in spontaneously diabetic Kir6.2[ΔN2-30] mice may contribute to the associated hyperglycemia and severely blunted GSIS (Fig. 5*A*), as well as to decreased insulin content in these islets (Fig. 5*B*).

DISCUSSION

Spectrum of K_{ATP}-induced diabetes. While several animal models of K_{ATP} underactivity have been generated (29–33), the only animal model of K_{ATP} "gain-of-function" is the Kir6.2[ΔN2-30] transgenic mice. We have generated seven transgenic founder mice expressing ATP-insensitive K_{ATP} channels in their β-cells under control of the RIP I. In the previous study, we described profound NDM in all offspring of the A–C and E founder mice (16). F1 mice were severely diabetic (blood glucose >600 mg/dl), ketoacidotic, and hypoinsulinemic within ~3 days of birth. Only 2% of transgenic mice that were born survived to weaning (day 21), with the rest dying within 1 week of birth. These mice strikingly modeled and predicted the phenotype of permanent NDM resulting from similar K_{ATP} mutations in humans (3). In the current study, we examined additional Kir6.2[ΔN2-30] founder mouse lines D, F, and G. In sharp contrast to the severely neonatally diabetic lines, most of these mice survive past weaning (day 21) despite clear evidence for functional expression of the transgene. However, disruption of glucose handling is evidenced as both glucose intolerance in nondiabetic adult mice and severe, spontaneous diabetes in a subpopulation of mice at or shortly after weaning.

Several lines of evidence implicate "overactive" β-cell K_{ATP} as underlying the hyperglycemia in these mouse lines. First, a diabetic phenotype was never observed in control littermate mice. Second, whole-animal studies establish a decreased glucose-to-insulin ratio in the blood and a reduction of insulin release in vivo in response to elevation of blood glucose. Third, pancreatic islet size, distribution, and morphology was unaltered in glucose-intolerant transgenic mice. Fourth, insulin content was unchanged in the transgenic islet. Finally, the decrease in ATP sensitivity of K_{ATP} from transgenic β-cells is commensurate with the observed suppression of GSIS. Thus, the animal phenotype in these Kir6.2[ΔN2-30] mice appears to result solely from a failure of insulin secretion as a result of increased K_{ATP} activity in the β-cell.

Cellular consequences of transgenic expression. We have previously described the phenotype of transgenic mice expressing a dominant-negative Kir6.2 subunit under

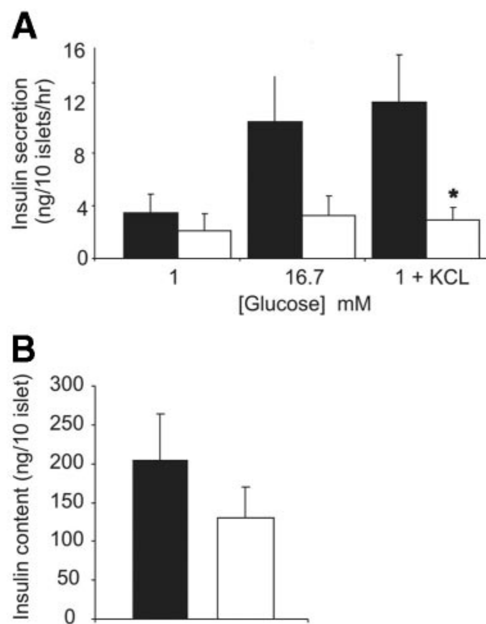

FIG. 5. Severe and overt diabetes in a subpopulation of transgenic mice around weaning. *A*: GSIS from islets isolated from spontaneously diabetic transgenic mice (□; n = 5 mice, aged 30–60 days, fed blood glucose >600 mg/dl) and control littermate mice (■; n = 6 mice, aged 30–60 days, fed blood glucose 119.3 ± 2.3 mg/dl). *B*: Total insulin content from nondiabetic control littermates (n = 25 islets from two mice) and spontaneously diabetic transgenic mice (n = 25 islets from two mice). All mice were fed a normal diet. Data represent means ± SE. *P < 0.05, unpaired Student's t test.

control of the same transgenic promoter (33–35). In contrast to Kir6.2[ΔN2-30] mice, the dominant-negative Kir6.2 transgenic mice (Kir6.2[AAA]) exhibit reduced β-cell K_{ATP} activity and are predictably hyperinsulinemic with normal islet morphology, even though the expression of the transgenic protein in Kir6.2[AAA] islets is at least as high as in the present animals. We can therefore reasonably exclude an artifactual effect of Kir6.2 (or GFP) overexpression as underlying the diabetic phenotype in our current study.

In the profoundly neonatally diabetic lines (A–C and E), a substantial number of green fluorescing transgenic β-cells were detected (16). In the present animals (lines D, F, and G), it is clear that not all β-cells in the islet express the transgene at visible levels (Fig. 1). Such mosaic expression is a common feature of transgenic mice, and it seems likely that differential transgene expression may account for the variable phenotype among these founder lines.

In humans, different Kir6.2 mutations will cause different degrees of loss of ATP sensitivity, but the expression of the mutation should be more or less uniform in all β-cells. Pancreatic islets function as electrical syncititia (36), and β-cells within the islet are well coupled to one another through gap junctions. If a few β-cells within an islet are expressing ATP-insensitive channels, the effect will be averaged across the whole islet. The proof of this principle was recently established in transgenic islets from Kir6.2[AAA] mice (35). In these islets, ~30% of β-cells express normal K_{ATP} channels, but the remaining ~70% express no functional K_{ATP} channels. Isolated β-cells with no K_{ATP} channels (i.e., expressing Kir6.2[AAA]) are glucose insensitive and hypersecreting. However, in the intact islet, the normal K_{ATP} channels in the wild-type cells (~30%) can still hyperpolarize the membrane potential across the islet and terminate insulin secretion. This means that in the present case of Kir6.2[ΔN2-30] mice, we can expect that the ATP insensitivity of transgenic channels will be averaged across the islet, and so the severity of the effect will depend on the average level of expression in all β-cells.

Implications for human NDM. What are the implications of the present findings for the etiology of K_{ATP}-induced diabetes, and can these mice inform the human disease? First of all, it is clear from both the previous (16) and present studies that the animals can be severely diabetic before any significant loss of β-cells or disruption of islet architecture has occurred. In the neonatally diabetic lines, mice were severely diabetic by day 3, yet islet architecture was often normal at this time (16). Similarly, in glucose-intolerant animals, islet architecture is normal and insulin

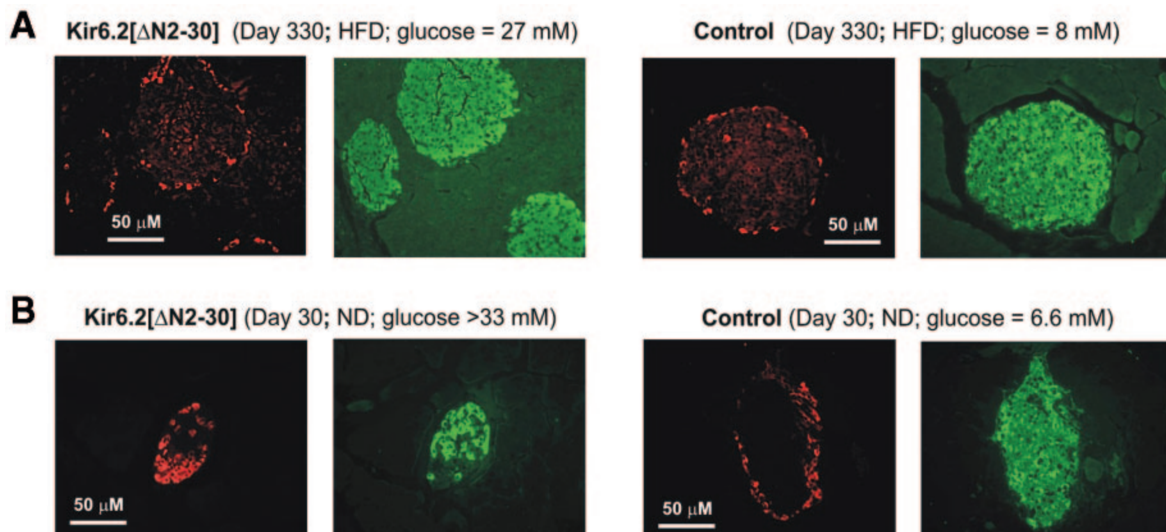

FIG. 6. Histological analysis of pancreatic islets from Kir6.2[ΔN2-30] and control mice. *A*: Immunostaining of pancreatic sections from an adult glucose-intolerant Kir6.2[ΔN2-30] mouse (*left*) and control littermate (*right*) on an HFD. Immunofluorescence with the anti-glucagon antibody is shown in red and staining with the anti-insulin antibody in green. *B*: Immunostaining of consecutive pancreatic sections from a spontaneously diabetic mouse (day 30) with an anti-glucagon (red) or anti-insulin antibody (green). Fed blood glucose 120 mg/dl (control) and >600 mg/dl (Kir6.2[ΔN2-30]). Mice were on a normal diet. Magnification: ×20, *left panels*; ×40, *right panels*.

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content is normal (Fig. 6). These results argue well for preservation of islet morphology and insulin content in NDM in humans, at least at the earliest stage of the disease. Clearly, sulfonylurea sensitivity can be maintained, since successful weaning of humans with K_{ATP}-induced diabetes onto sulfonylureas has been performed in a number of cases (11). However, our results also suggest that once a diabetic state persists, there is a loss of insulin content (Fig. 5) and α-cell infiltration into the islet, possibly a result of glucose toxicity (Fig. 6). Whether such progression might be halted by stimulation with sulfonylureas and normalization of blood glucose levels is unknown, although further experiments with these animal models should provide insight.

Genetic studies implicate altered K_{ATP} activity in a broad spectrum of human diabetic phenotypes with variable onset and severity (3,7,8,17). In all cases, K_{ATP} "gain-of-function" is postulated to underlie β-cell dysfunction. Consistent with the genetic studies, transgenic models of K_{ATP} overactivity recapitulate a similar spectrum of diabetic phenotypes. At one extreme, we reported a severe NDM in Kir6.2[ΔN2-30] transgenic mice (16). In the present study, we now describe transgenic mice demonstrating milder, later-developing forms of K_{ATP}-induced diabetes characterized by glucose intolerance in adult transgenic mice and development of overt diabetes in a subpopulation of mice. Importantly, impaired GSIS can account for the diabetic phenotypes. These Kir6.2[ΔN30] transgenic mice should therefore be useful as models of the variable forms of K_{ATP}-induced diabetes in which overactive β-cell K_{ATP} contributes to different forms of hyperglycemia: from the monogenic disorder of NDM to a mild risk factor in the complex etiology of type 2 diabetes (E23K) and gestational diabetes (the C42R mutation and E23K polymorphism).

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