Parasympathetic Innervation and Function of Endocrine Pancreas Requires the Glial Cell Line–Derived Factor Family Receptor α2 (GFRα2)

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Vagal parasympathetic input to the islets of Langerhans is a regulator of islet hormone secretion, but factors promoting parasympathetic islet innervation are unknown. Neurturin signaling via glial cell line–derived neurotrophic factor family receptor α2 (GFRα2) has been demonstrated to be essential for the development of subsets of parasympathetic and enteric neurons. Here, we show that the parasympathetic nerve fibers and glial cells within and around the islets express GFRα2 and that islet parasympathetic innervation in GFRα2 knockout (KO) mice is reduced profoundly. In wild-type mice, neuroglucopenic stress produced a robust increase in plasma levels of islet hormones. In the GFRα2-KO mice, however, pancreatic polypeptide and insulin responses were completely lost and glucagon response was markedly impaired. Islet morphology and sympathetic innervation, as well as basal secretions of the islet hormones, were unaffected. Moreover, a glucose tolerance test failed to reveal differences between the genotypes, indicating that direct glucose-stimulated insulin secretion was not affected by GFRα2 deficiency. These results show that GFRα2 signaling is needed for development of the parasympathetic islet innervation that is critical for vagally induced hormone secretion. The GFRα2-KO mouse represents a useful model to study the role of parasympathetic innervation of the endocrine pancreas in glucose homeostasis. *Diabetes* 54:1324–1330, 2005

Endocrine cells in the islets of Langerhans are well innervated by sympathetic, parasympathetic, and sensory nerve fibers. The parasympathetic branch is thought to be a regulator of the physiological islet hormone secretion (1–4). The parasympathetic fibers in the endocrine pancreas originate from neurons in the intrapancreatic ganglia (5) that receive preganglionic input from the brainstem via the vagus nerve, as well as direct input from enteric (6) and other intrapancreatic neurons (7). Activation of the vagus nerve is known to stimulate the secretion of insulin and other pancreatic hormones, although the relative contributions of noncholinergic parasympathetic neurotransmitters and the enteropancreatic projection to islet hormone secretion remain elusive (3). Meal-induced insulin secretion is traditionally divided into a preabsorptive or cephalic phase that is vagally mediated (3,4) and a subsequent and much larger postabsorptive or postprandial phase that is thought to be mainly controlled by circulating glucose levels. However, several studies (8–10) suggest that parasympathetic regulation of postprandial insulin secretion may be more important than previously believed.

Sympathetic innervation of the pancreatic islets is promoted by the nerve growth factor (11,12). Factors that control the development and maintenance of islet parasympathetic innervation, by contrast, are poorly known. Neurturin, a member of the glial cell line–derived factor family, signals through glial cell line–derived factor family receptor α2 (GFRα2) and has been found to be essential for the development of enteric and parasympathetic innervation of several target tissues (13). GFRα2 knockout (KO) mice have various neuronal deficits in cholinergic innervation along the alimentary tract, including the salivary and exocrine pancreatic glands and the small intestine (14). Here, we have combined immunohistochemical analysis with physiological tests to study the in vivo role of GFRα2 signaling in endocrine pancreas innervation and islet cell function.

RESEARCH DESIGN AND METHODS

Immunohistological analysis of islet innervation. GFRα2-KO and wild-type littermates in an F1 hybrid background (C57BL/6 × 129S2) were obtained and genotyped as described earlier (14,15). All animal experiments were approved by the Animal Research Ethics Committee at the University of Helsinki. Adult wild-type and GFRα2-KO littermate mice were anesthetized with chloral hydrate and perfused transcardially with 4% paraformaldehyde in PBS, pH 7.5. The pancreas was removed and postfixed at 4°C for 2–3 h or overnight (depending on antibody used), cryoprotected in sucrose, and cut into 10- to 20-μm sections that were stained using standard immunofluorescence techniques. Primary polyclonal antibodies were against pancreatic polypeptide (PP) (guinea pig; Linco Research), somatostatin and glucagon (rabbit; Affiniti), insulin (guinea pig; Abcam), GFRα2 (goat; R&D Systems), vesicular acetylcholine transporter (VAChT) (goat; Chemicon, or rabbit; Phoenix Pharmaceuticals, Mountain View, CA), tyrosine hydroxylase (TH) (rabbit and sheep; Chemicon), S100β (rabbit; Swant), and vasoactive intestinal peptide (VIP) (rabbit; Progen). Donkey secondary antibodies were from

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2-DG, 2-deoxyglucose; GFRα2, glial cell line–derived factor family receptor α2; PP, pancreatic polypeptide; TH, tyrosine hydroxylase; VAChT, vesicular acetylcholine transporter; VIP, vasoactive intestinal peptide.

Jackson ImmunoResearch (Cy2, Cy3, or TexasRed label) and Molecular Probes (Alexa488 or Alexa555 label). For colocalization analysis, sections were scanned with a Zeiss confocal microscope, and maximum intensity projections were made using the LSM5 software. Around 20 optical sections were used to make each image.

To measure islet innervation density and islet area, ~40 islet sections per animal ($n = 5$ mice per genotype) were used, and staining was analyzed from the serial sections (~200 μm apart) covering the whole pancreas. Each islet was delineated, and artifacts (and TH^+ cell bodies inside the islets) were removed from digital microscopy images using the Adobe Photoshop 6.0 program. Islet cross-section areas (μm^2) and the number of $VAChT^+$, VIP^+, and TH^+ pixels in each islet cross-section area were determined using the ImagePro 4.0 program (Media Cybernetics, Silver Spring, MD). Innervation density was expressed as the percentage of islet profiles with a given density of immunopositive varicosities per islet area (arbitrary units). An islet size distribution histogram was prepared using Microsoft Excel.

Vagally stimulated islet hormone secretion. For vagal stimulation of islet hormone secretion, 2-deoxyglucose (2-DG) (Sigma; dissolved in saline) was injected intraperitoneally (6 mmol/kg body wt) in adult sex-matched wild-type and GFRα2-KO littermate mice. Separate animals were used for each hormone measurement. For PP, mice were fasted overnight and blood samples (100 μl) were collected from the tail 1 week before and at 15 and 30 min after 2-DG stimulation. For insulin and glucagon measurements, nonfasted animals were used and tail blood samples (40 and 100 μl, respectively) were collected just before and 10 min after 2-DG injection, a time point previously reported for peak insulin level (16). Insulin measurement was repeated on 2 consecutive weeks, and an aliquot of the blood samples was used to study plasma glucose levels from the same group. Plasma was separated by brief centrifugation at 4°C and stored at −20°C until analysis. PP concentration was measured by radioimmunoassay using guinea pig anti-rat PP antibody, ^{125}I-labeled rat PP as a tracer, and rat PP as a standard, followed by free and bound radioactivity separation by goat anti–guinea pig IgG antibody (Linco Research). Heat-inactivated plasma from untreated wild-type mice was added to the standards to the same volume as in the samples. Insulin and glucagon were measured using mouse insulin enzyme-linked immunosorbent assay and glucagon RIA kits (Linco Research), respectively, according to the manufacturer's protocols.

Glucose tolerance test. After collection of baseline blood samples (40 μl from tail vein), D-glucose (2 mg/g; Sigma) was administered intraperitoneally to 3- to 6-month-old mice fasted overnight. Blood samples (40 μl) for glucose and insulin measurements were collected from the tail at 20 and 120 min, and additional samples (20 μl) were collected for glucose at 10 and 60 min after injection. All blood samples were chilled on ice, and plasma was separated by brief centrifugation and stored at −70°C until analysis.

Statistical analysis. Results are expressed as means ± SE. Repeated-measure ANOVA was used to evaluate differences in 2-DG–induced secretion of islet hormones between wild-type and GFRα2-KO mice. Post hoc comparisons were carried out using the Bonferroni test. Data from all other measurements were compared using the two-tailed Student's t test, assuming unequal variance. $P < 0.05$ was considered statistically significant.

RESULTS

Localization of GFRα2 in islets of Langerhans. Consistent with our previous results of the GFRα2 protein being expressed in intrapancreatic parasympathetic neurons and $S100\beta^+$ Schwann cells (14), the GFRα2 protein was seen in all $S100\beta^+$ Schwann cells encircling the islets (Fig. 1*A–C*), as well as in most (if not all) thin fibers and varicosities labeled by the parasympathetic markers VAChT (Fig. 1*D–F*) or VIP (Fig. 1*G–I*). Consistent with this, most VIP^+ nerve fibers were immunoreactive for VAChT, and most $VAChT^+$ fibers were VIP^+ (Fig. 1*J–L*), although individual varicosities appeared to express the two markers at different levels. All sympathetic (TH^+) nerve fibers in the islets were $GFR\alpha2^-$ (Fig. 1*M–O*). Taken together, these results indicate that GFRα2 is expressed in Schwann cells and in the parasympathetic nerve fibers of pancreatic islets.

Reduced islet parasympathetic innervation in GFRα2-KO mice. We used the neuronal markers VIP and VAChT to study whether the parasympathetic innervation of the endocrine pancreas is affected in GFRα2-KO mice. In the wild-type pancreas, all islets showed moderate or high intensity of VIP and VAChT staining in varicose nerve fibers occupying the whole islet area (Fig. 2*A* and *D*; see also Fig. 1*C* in the online appendix at http://diabetes.diabetesjournals.org). Compared with wild-type islets, the density of parasympathetic innervation in GFRα2-KO islets was profoundly reduced (Fig. 2*B* and *E*; see also Fig. 1*D* in the online appendix). In most GFRα2-KO islets, the innervation was either clearly reduced or completely absent. Remarkably, some islets in GFRα2-KO mice appeared to have normal density of VIP^+ and $VAChT^+$ fibers (Fig. 2*B*). Estimation of the innervation density as average number of positive pixels per islet area revealed a 56% reduction of VIP^+ (WT 4.7 ± 0.7 vs. KO 2.1 ± 0.5, $P = 0.02$, $n = 5$) and an 80% reduction of $VAChT^+$ (WT 4.3 ± 0.8 vs. KO 0.9 ± 0.3, $P = 0.01$, $n = 5$) nerve fibers in GFRα2-KO mice compared with the wild-type controls. In wild-type animals, islet profiles invariably had some VIP^+ and $VAChT^+$ varicosities, whereas in GFRα2-KO mice, ~19% (55 of 295) of islet sections had no VIP labeling and ~35% (66 of 187) had no VAChT labeling (Fig. 2*C* and *F*). As in the exocrine pancreas (14), the density of sympathetic TH^+ innervation in the islets was not different between the genotypes (WT 4.7 ± 0.3 vs. KO 5.8 ± 1.4, $P = 0.5$, $n = 3$) (see Fig. 2 in the online appendix at http://diabetes.diabetesjournals.org). The islet size distribution appeared similar between the genotypes (see Fig. 3 in the online appendix), although the mean islet cross-section area was slightly smaller in GFRα2-KO animals (WT 24,500 ± 800 μm^2 vs. KO 21,900 ± 600 μm^2, $P = 0.02$, $n = 5$). The distribution of insulin cells in the central part of the islets, as well as PP, somatostatin, and glucagon cells in the periphery, seemed unaffected in the KO mice (see Fig. 1*A–D* in the online appendix; data not shown).

GFRα2-KO mice lack vagally stimulated PP and insulin secretion and have a severely reduced glucagon response. Parasympathetic cholinergic activation via the vagal nerve is known to mediate physiological secretion of islet hormones, including PP and insulin (1,3). This vagally mediated secretion can also be stimulated by 2-DG–induced neuroglucopenia (16,17). To test the physiological consequence of reduced islet parasympathetic innervation in GFRα2-KO mice, we measured the secretion of PP, insulin, and glucagon after 2-DG administration. Plasma values for these variables were similar between sexes and thus were pooled together. Because a sex difference has previously been demonstrated in 2-DG–stimulated glucagon secretion (18), only male mice were used for glucagon measurements. Basal PP levels were not significantly different between wild-type and GFRα2-KO mice (Fig. 3*A*). 2-DG stimulation significantly increased PP secretion in wild-type mice (baseline 160 ± 20 pg/ml, stimulated 270 ± 20 pg/ml, $n = 7$, $P < 0.01$) as expected, but failed to induce any increase in GFRα2-KO mice (baseline 160 ± 30 pg/ml, stimulated 140 ± 20 pg/ml, $n = 6$) (Fig. 3*A*). At 30 min, PP concentration had returned close to baseline levels in wild-type animals and was lower than baseline in GFRα2-KO mice (WT 180 ± 30 pg/ml, KO 130 ± 20 pg/ml, $n = 6$). Similarly, administration of 2-DG significantly increased insulin secretion in wild-type mice (baseline 1.3 ± 0.2 ng/ml, stimulated 5.8 ± 1.4 ng/ml, $n = 5$, $P <$

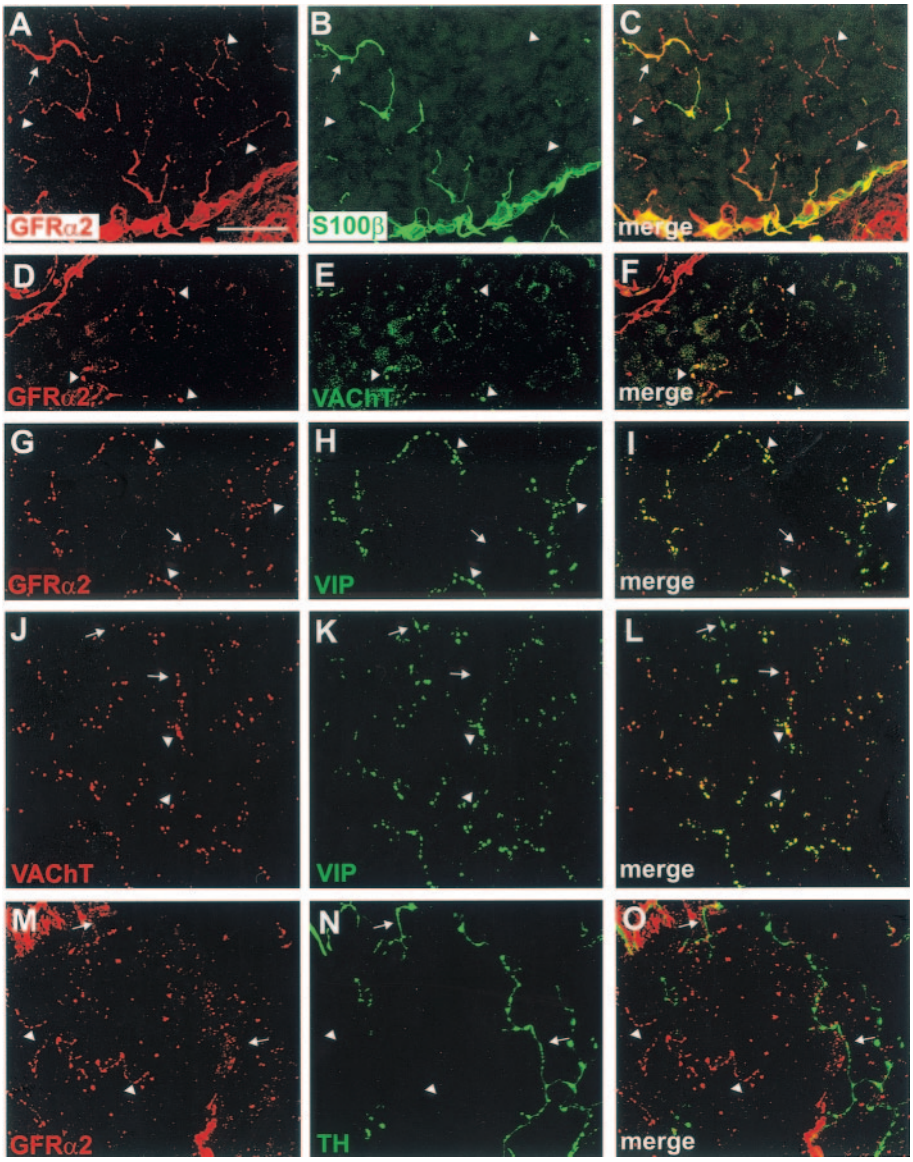

FIG. 1. Localization of the GFRα2 protein in Schwann cells and parasympathetic nerve fibers in the wild-type mouse endocrine pancreas. *A–C*: Colocalization (*C*, yellow) of GFRα2 (*A*, red) and S100β (*B*, green) in islets of Langerhans. The GFRα2 protein is localized in S100β$^+$ Schwann cells encircling the islets. Some S100β- and GFRα2-containing glial processes are also present in the center of the islets (small arrow). Arrowheads indicate thin and varicose GFRα2$^+$ and S100β$^-$ nerve fiber distributed randomly in the center of the islet. *D–I*: Most GFRα2$^+$ (*D* and *G*, red) thin varicose nerve fibers (arrowheads) express the parasympathetic markers VAChT (*E*, green) and VIP (*H*, green). Colocalization is seen in yellow (*F* and *I*). The arrow points indicate GFRα2$^+$ varicosities that are VIP$^-$. *J–K*: The parasympathetic markers, VAChT (*J*, red) and VIP (*K*, green), show similar varicose staining and are colocalized (*L*, yellow) in most nerve fibers in the islets (arrowheads). Note that some of the varicosities contain either VIP or VAChT but not both of the markers (arrows). *M–O*: GFRα2 (*M*, red) (arrowheads) is not expressed in sympathetic nerve fibers (arrows) labeled with TH (*N*, green) in the islets. Bar equals 50 μm.

0.01), but not in GFRα2-KO mice (baseline 1.3 ± 0.3 ng/ml, stimulated 1.3 ± 0.2 ng/ml, $n = 5$) (Fig. 3*B*). Glucagon response after 2-DG stimulation was prominent in wild-type animals (baseline 81 ± 4 pg/ml, stimulated 620 ± 70 pg/ml, $n = 3$, $P < 0.001$), but the response was significantly ($P < 0.01$) reduced in GFRα2-KO mice (baseline 83 ± 11 pg/ml, stimulated 260 ± 30 pg/ml, $n = 4$) (Fig. 3*C*). As previously reported (14), baseline glucose levels of nonfasted animals were not different between genotypes ($n = 5$ in both genotypes) (Fig. 3*D*). Administration of 2-DG elevated plasma glucose levels in wild-type mice (baseline 182 ± 7 mg/dl, stimulated 365 ± 6 mg/dl, $n = 5$), as shown earlier (16), as well as in GFRα2-KO mice (baseline 188 ± 10 mg/dl, stimulated 374 ± 23 mg/dl, $n = 5$) (Fig. 3*D*).

Normal systemic glucose tolerance in GFRα2-KO mice. To further address the role of the GFRα2 receptor in glucose homeostasis, we carried out an intraperitoneal glucose tolerance test using D-glucose (2 mg/g body wt). Plasma glucose levels increased after D-glucose administration and declined close to baseline levels by 120 min in both wild-type and GFRα2-KO mice (Fig. 4*A*). The insulin response to systemic glucose load was similar between the groups (Fig. 4*B*).

DISCUSSION

Localization of the GFRα2 protein in the endocrine pancreas. Intrapancreatic nerves are formed by networks of unmyelinated nerve fibers consisting of thin axons and associated Schwann cells (19,20). We show here that all S100β$^+$ "peri-insular" Schwann cells in the endocrine pancreas express GFRα2. The role of GFRα2 in these glial cells is unknown and requires further study. In agreement with expression of GFRα2 in most, if not all, VIP$^+$ intrapancreatic neurons (14), apparently all VIP$^+$ thin nerve fibers in the islets were GFRα2$^+$. Similarly, all VAChT$^+$ fibers in the islets appeared to express GFRα2. On the other hand, all GFRα2$^+$ fibers were TH$^-$, indicating that GFRα2 is expressed in parasympathetic but not sympathetic axons within the endocrine pancreas. Consistent with this, and similarly to the exocrine pancreas (14), we demonstrate that GFRα2 is not required for sympathetic innervation of islets of Langerhans.

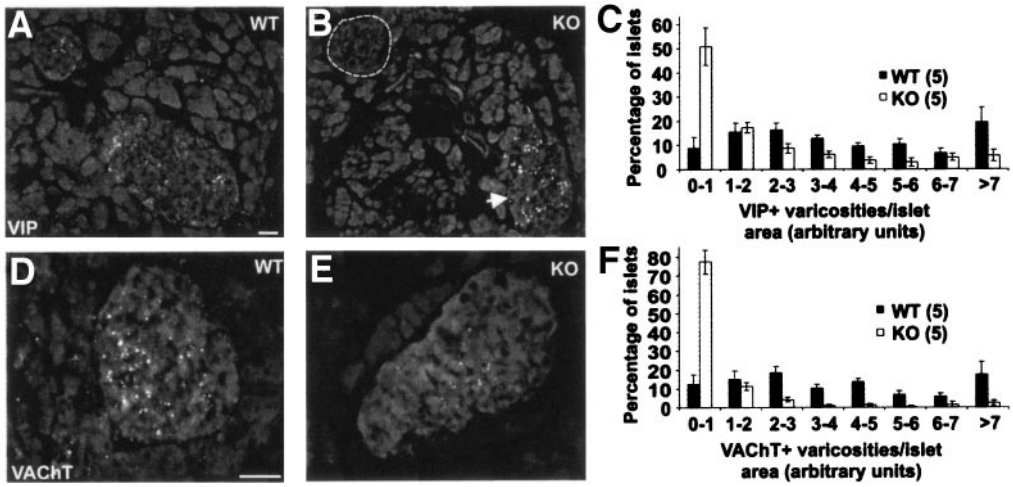

FIG. 2. Reduced cholinergic innervation of islets of Langerhans in the GFRα2-KO mouse pancreas. *A*: VIP immunohistochemistry stains several varicose nerve fibers in wild-type islets. *B*: In the GFRα2-KO pancreas, many islet profiles are completely devoid of parasympathetic fibers (circled area in *B*), but some islets have apparently normal density of VIP$^+$ innervation (arrow). *D* and *E*: Density of VAChT$^+$ varicosities is reduced in GFRα2-KO islets. Bars equal 50 μm. *C* and *F*: Density histograms of islet parasympathetic innervation shown as percentage of islet profiles with a given density of immunopositive varicosities per islet area (arbitrary units). In the wild-type pancreas (■), most islet profiles have moderate to high density of varicose, VIP$^+$ (*C*), and VAChT$^+$ (*F*) nerve fibers. In contrast, the number of islet profiles with low density of nerve fibers is greatly increased in the GFRα2-KO pancreas (□).

Indirect evidence suggests that islet sensory innervation is GFRα2$^-$. A vast majority of sensory neurons that project into the pancreas are calcitonin gene–related peptide positive (21,22), whereas virtually all spinal sensory neurons that express GFRα2 are CGRP$^-$ and do not project into the pancreas (M.S.A., unpublished data) (23). In addition, although some sensory neurons in the nodose ganglion project into the islets (24), few nodose ganglion neurons express GFRα2 (25). Thus, the afferent sensory innervation of the islets is predicted to be intact in the GFRα2-KO mice.

We show that islet endocrine cells do not express GFRα2 and are distributed similarly in the wild-type and the GFRα2-KO pancreas, indicating that GFRα2 is not necessary for the segregation of islet endocrine cells. Furthermore, the comparable islet size distribution between wild-type and GFRα2-KO mice suggest that islet development does not require the GFRα2 receptor. Although mean islet profile size was slightly (~10%) reduced in the adult GFRα2-KO mice, this can be explained by the overall reduction in their body weight and organ size (15).

Reduced parasympathetic innervation of the endocrine pancreas in GFRα2-KO mice. In a previous study, we noted apparently normal acetylcholinesterase staining in some islets of the GFRα2-KO mice, in contrast to a virtually complete lack of acetylcholinesterase-positive nerve fibers in the exocrine pancreas (14). Using the more specific parasympathetic markers VIP and VAChT, we demonstrate here that parasympathetic innervation is profoundly reduced in GFRα2-KO mice compared with their wild-type littermates. However, some 20% of VAChT$^+$ and 44% of VIP$^+$ nerve fibers remain in GFRα2-KO islets. Some of these fibers may belong to the subpopulation of ~15% of intrapancreatic parasympathetic neurons that do not express GFRα2 in wild-type mice and are retained in GFRα2-KO mice (14). It is also possible that some of the

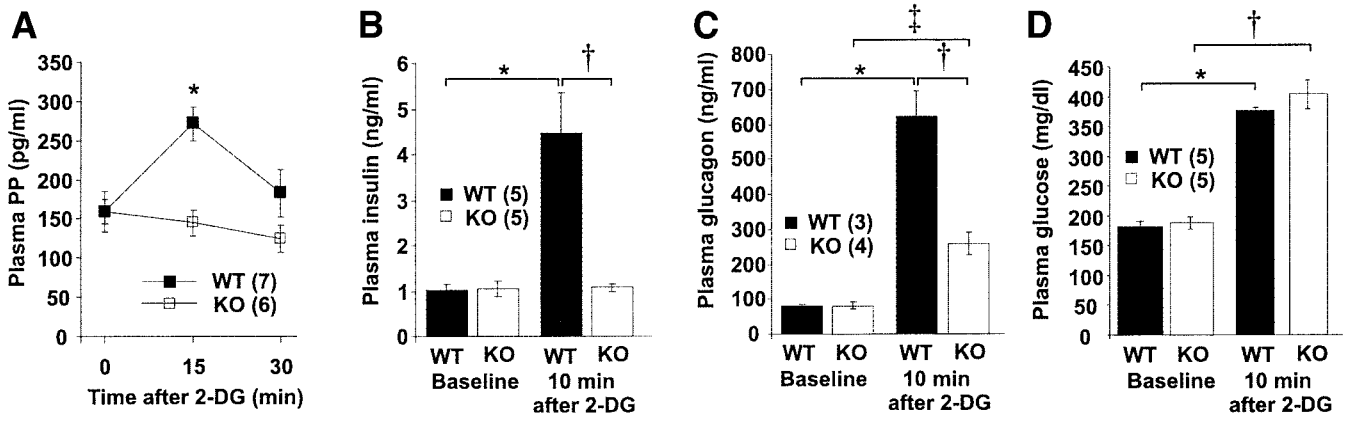

FIG. 3. Loss of vagally stimulated secretion of PP and insulin and diminished glucagon response in GFRα2-KO mice. *A*: In wild-type animals, 2-DG, a central vagal stimulant, induces secretion of PP. **P* < 0.01 for WT vs. KO. After 30 min, plasma PP returned close to baseline levels in wild-type mice. In GFRα2-KO mice, 2-DG–induced PP secretion is absent. *B*: 2-DG induces secretion of insulin in wild-type but not GFRα2-KO mice. **P* < 0.01 for WT baseline vs. 2-DG; †*P* < 0.001 for WT vs. KO. *C*: Secretion of glucagon is profoundly reduced in GFRα2-KO mice after 2-DG stimulation. **P* < 0.001 for WT baseline vs. 2-DG; †*P* < 0.01 WT vs. KO; ‡*P* < 0.05 for KO baseline vs. 2-DG. *D*: Serum glucose levels before and after 2-DG. The glucose levels are measured from the same blood samples as in *B*. **P* < 0.001 for WT baseline vs. 2-DG; †*P* < 0.001 for KO baseline vs. 2-DG. The number of animals in each group is presented in parentheses.

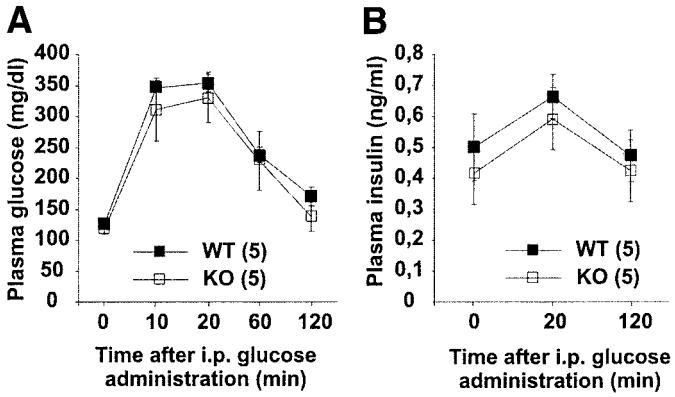

FIG. 4. Normal systemic glucose tolerance and insulin response in GFRα2-KO mice. Plasma glucose (*A*) and insulin (*B*) values are similar for wild-type and GFRα2-KO mice before and after an intraperitoneally administered glucose load (2 mg/g body wt).

remaining VAChT$^+$ and VIP$^+$ innervation in the islets of GFRα2-KO mice is of enteric origin (6).

The reason for the disparity between the quantitative immunohistochemistry of VIP$^+$ versus VAChT$^+$ fibers remains unclear, although there are several possible explanations. First, it is possible that the subpopulation of VIP$^+$ fibers that appeared VAChT$^-$ would not be parasympathetic and therefore did not disappear in the GFRα2-KO mice. In rat pancreas, the VIP-containing nerve fibers are resistant to the sensory neurotoxin capsaicin and have an almost entirely intrinsic origin (26). Thus, it seems unlikely that the VIP$^+$ and VAChT$^-$ fibers would be sensory, although we have not excluded the possibility that VIP would be upregulated in the islet sensory fibers in the GFRα2-KO mice. Double staining for TH and VIP indicates that the VIP fibers in GFRα2-KO mouse pancreas are not sympathetic (data not shown). Second, some of the remaining VIP fibers might originate from noncholinergic intrapancreatic parasympathetic neurons, but the extent of colocalization of VIP with cholinergic markers in mouse intrapancreatic neurons remains to be studied. Finally, given that the GFRα2 ligands can induce cholinergic differentiation in other neurons (27,28), another explanation would be downregulation of VAChT expression in the remaining VIP$^+$ parasympathetic fibers in the GFRα2-KO mice.

Complete loss of vagally stimulated secretion of PP and insulin and severely reduced secretion of glucagon in GFRα2-KO mice. We show here that GFRα2-KO mice fail to secrete PP and insulin after 2-DG administration. 2-DG is known to induce a glucopenic stress that stimulates secretion of islet hormones via the vagus nerve (17). Baseline plasma glucose levels were similar in the wild-type and GFRα2-KO mice and increased equally after 2-DG, indicating that the stimulation was comparable in both groups. Baseline PP and insulin levels were also similar between the genotypes, consistent with previous findings that parasympathetic blockage (by atropine or vagotomy) does not change baseline islet hormone levels (4,29,30). Regarding the PP secretion, the hormone levels were significantly elevated 15 min after 2-DG injection in the wild-type mice, returning to baseline levels by 30 min, as described previously (16,17). By contrast, PP levels remained at baseline levels at both the 15- and 30-min time points in the GFRα2-KO mice. This indicates that 2-DG–stimulated PP secretion is absent and not merely delayed in GFRα2-KO mice. Vagally mediated secretion of PP and insulin is predominantly cholinergic (1), and 2-DG fails to induce secretion of PP and insulin in mice pretreated with cholinergic antagonist atropine (17,31). Thus, the complete lack of PP and insulin responses in the GFRα2-KO mice can be explained by reduced cholinergic innervation to the islets, assuming that the neurons responsible for the remaining islet parasympathetic innervation do not receive vagal input or are functionally inactive.

In contrast to the lack of vagally stimulated PP and insulin secretion, the secretion of glucagon after vagal stimulation was not completely abolished in GFRα2-KO mice, albeit it was markedly reduced compared with wild-type mice. Previous studies have shown that sympathetic blockers are required in addition to atropine to fully inhibit the glucagon response to glucopenia (17). This suggests that the intact sympathetic innervation of the islets mediates the remaining (~33%) glucagon response after 2-DG administration in GFRα2-KO mice. Transplantation of the islets of Langerhans to the liver is one potential therapeutic strategy for treating type 1 diabetes. However, a major drawback of this strategy is hypoglycemia due to severely impaired glucagon response of the islet transplants (32). Local nerve growth factor application has already been successfully used in an animal model of diabetes to improve sympathetic reinnervation and graft function (33). Thus, our findings that GFRα2 is needed for islet parasympathetic innervation of the islets and for the glucagon response during glucopenic stress suggest that exogenous application of GFRα2 ligands (including neurturin) could augment parasympathetic re-innervation and function of the islet grafts.

Normal glucose tolerance in GFRα2-KO mice. We show that a systemic glucose load leads to a similar glucose tolerance and insulin response in GFRα2-KO mice as in wild-type animals. This indicates that the ability of the GFRα2-KO mice to metabolize glucose from the blood and to secrete insulin from their β-cells upon blood glucose elevation is unaffected. Whether the mice show abnormalities in more physiological tests of glucose metabolism, including fasting and meal-induced islet hormone secretion, remains to be determined.

We show that 2-DG–induced hyperglycemia is similar in GFRα2-KO mice as in wild-type controls despite marked impairment of glucagon secretion. This indicates that the hyperglycemia cannot be due to concomitant 2-DG–induced glucagon secretion. Our results are consistent with findings that the 2-DG–induced hyperglycemia is a combined result of stimulation of the hepatic glycogenolysis by epinephrine from the adrenal gland and possibly by the sympathetic nerves in the liver (34,35).

Implications to type 2 diabetes. An array of metabolic, neural, and hormonal factors modulate primary glucose-induced insulin secretion (36). Insulin secretion in response to systemic glucose (or other stimuli) is biphasic, with an early burst in insulin release followed by a gradual increase over several hours (37,38). The cephalic phase of insulin secretion is thought to play an important role in preserving normal glucose tolerance (39,40), and restoration of this phase in type 2 diabetic patients improves

glucose tolerance (41). It is unknown whether an impaired preabsorptive vagal stimulus contributes to the pathogenesis of the first-phase insulin secretion deficit in type 2 diabetes (4). Ahren and Holst (40) suggested that a failure of islet parasympathetic innervation may be involved in the development of glucose intolerance and that augmentation of neural-induced insulin secretion might be a target for treatment of islet dysfunction in diabetes. In obese animal models, hyperinsulinemia is due to an increased vagal cholinergic tone (42,43), and cholinergic activation with pyridostigmine increases total insulin output stimulated by oral glucose in obese but not normal-weight women (44). Thus, cholinergic stimulation may help improve secretion of insulin (and other islet hormones) and thus glucose homeostasis in patients with type 2 diabetes.

In conclusion, our results show that GFRα2 signaling is needed for development of the islet parasympathetic innervation that is critical for vagally induced secretion of pancreatic hormones. GFRα2-KO mice represent a novel and unique model for studying the role of parasympathetic innervation of the endocrine pancreas in glucose homeostasis and are also likely to be a useful animal model for studies on the role of cholinergic innervation of the pancreas in the pathogenesis of type 2 diabetes.

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