The Role of Voltage-Gated Potassium Channels Kv2.1 and Kv2.2 in the Regulation of Insulin and Somatostatin Release from Pancreatic Islets[S]

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ABSTRACT

The voltage-gated potassium channels Kv2.1 and Kv2.2 are highly expressed in pancreatic islets, yet their contribution to islet hormone secretion is not fully understood. Here we investigate the role of Kv2 channels in pancreatic islets using a combination of genetic and pharmacologic approaches. Pancreatic β-cells from Kv2.1$^{-/-}$ mice possess reduced Kv current and display greater glucose-stimulated insulin secretion (GSIS) relative to WT β-cells. Inhibition of Kv2.x channels with selective peptidyl [guangxitoxin-1E (GxTX-1E)] or small molecule (RY796) inhibitors enhances GSIS in isolated wild-type (WT) mouse and human islets, but not in islets from Kv2.1$^{-/-}$ mice. However, in WT mice neither inhibitor improved glucose tolerance in vivo. GxTX-1E and RY796 enhanced somatostatin release in isolated human and mouse islets and in situ perfused pancreata from WT and Kv2.1$^{-/-}$ mice. Kv2.2 silencing in mouse islets by adenovirus-small hairpin RNA (shRNA) specifically enhanced islet somatostatin, but not insulin, secretion. In mice lacking somatostatin receptor 5, GxTX-1E stimulated insulin secretion and improved glucose tolerance. Collectively, these data show that Kv2.1 regulates insulin secretion in β-cells and Kv2.2 modulates somatostatin release in δ-cells. Development of selective Kv2.1 inhibitors without cross inhibition of Kv2.2 may provide new avenues to promote GSIS for the treatment of type 2 diabetes.

Introduction

Pancreatic islets contain several types of highly interactive endocrine cells secreting specific hormones including insulin (β-cells), glucagon (α-cells), and somatostatin (δ-cells) (Muoio and Newgard, 2008; van Belle et al., 2011). Hormone release is tightly controlled by ion channels that regulate cell membrane potential and calcium influx (Rorsman, 1997; Kanno et al., 2002; Drews et al., 2010). Both members of the Kv2 voltage-gated potassium (Kv) channel family, Kv2.1 and Kv2.2, are expressed in pancreatic islets across several species (MacDonald et al., 2002, 2003; Yan et al., 2004; Herrington et al., 2005), with Kv2.1 being highly enriched in islet β-cells (MacDonald et al., 2002; MacDonald and Wheeler, 2003; Yan et al., 2004). Although germline null mutation of

Kv2.1 has been shown to promote islet insulin secretion in vitro and to lower blood glucose levels in vivo (Jacobson et al., 2007), pharmacologic studies with Kv2 inhibitors have not convincingly shown a role for Kv2 channels in regulating glucose stimulated insulin secretion (GSIS) in islets (Herrington et al., 2006; Braun et al., 2008), and, moreover the biologic role(s) of Kv2.2 in islets has not been assigned.

Kv channels are also expressed in somatostatin-secreting islet δ-cells (Göpel et al., 2000; Braun et al., 2009), although it remains unclear whether they mediate δ-cell membrane repolarization and glucose-stimulated somatostatin secretion, similar to their roles in β-cells (Braun et al., 2009). In this study, with the use of selective Kv2.x inhibitors and genetic tools, we present evidence supporting the idea that Kv2.1 plays an important role in regulating insulin secretion in β-cells, whereas Kv2.2 is critical for the control of somatostatin secretion in δ-cells. The increase in somatostatin levels caused by Kv2.x inhibitors provides a negative feedback on the β-cell that opposes the inhibitory effect of these agents on Kv2.1 channels to increase insulin release

ABBREVIATIONS: DMSO, dimethylsulfoxide; GSIS, glucose-stimulated insulin secretion; GxTX-1E, guangxitoxin-1E; IPGTT, intraperitoneal glucose tolerance test; RPMI medium, Roswell Park Memorial Institute medium; shRNA, small hairpin RNA; K$_V$ channel, voltage-gated potassium channel; SSTR5, somatostatin receptor subtype 5; WT, wild-type.

and lower blood glucose. Our studies suggest that development of selective Kv2.1 inhibitors may provide new avenues to promote GSIS for the treatment of type 2 diabetes.

Materials and Methods

Compounds. Guangxitoxin-1E (GxTX-1E) was purchased from Peptides International (Louisville, KY). RY796 was synthesized at Merck Research Laboratories. All other reagents were purchased from Sigma-Aldrich Chemicals (St. Louis, MO) unless otherwise specified.

Animals. Wild-type (WT) C57BL/6 mice were purchased from Taconic Farm (Germantown, NY). Kv2.1$^{-/-}$ mice (originally from Deltagen) were backcrossed onto a C57BL/6 background for six generations at Taconic Farm. Kv2.1$^{-/-}$ mice were produced through het × het breeding, and WT littermates were used as controls. Somatostatin receptor 5 knockout (SSTR5$^{-/-}$) mice, kindly provided by Dr. F.C. Brunicardi of Baylor College of Medicine (Houston, TX) (Wang et al., 2005), were backcrossed and bred using a scheme similar to the Kv2.1$^{-/-}$ mice. Mice used in this study were 2- to 6-month-old, age- and gender-matched littermates including both sexes. All mice were acclimated in our laboratory for at least 1 week before experiments, and were given free access to rodent normal chow (Teklad 7012) and water and housed on a 12-hour light/dark cycle. All animal experiments and procedures were approved by the Institutional Animal Care and Use Committee of Merck & Co.

Isolation of Pancreatic Islets and the Static GSIS Assay. Mouse islets were isolated by collagenase digestion and discontinuous Ficoll gradient separation (Lacy and Kostianovsky, 1967) and cultured overnight in RPMI 1640 medium with 11 mM glucose. Insulin secretion was determined by 1-hour static incubation in a 96-well format, as previously described elsewhere (Herrington et al., 2006).

Insulin Secretion in Perifused Mouse and Human Islets. Two independent batches of human islets (purity > 85%, viability > 90%) from normal subject cadaver organ donors (aged 20 and 58 years old, respectively) were obtained from the Islet Cell Resource Centers and the National Disease Resource Interchange (Philadelphia, PA) with appropriate consent and Merck review board approval for research use of human islets. Islets were handpicked and transferred to Roswell Park Memorial Institute (RPMI) medium (10% fetal calf serum, 5.5 mM glucose), and were cultured for at least 24 hours before use. Islets from WT and Kv2.1$^{-/-}$(Kcnb1$^{-/-}$) mice were also given an overnight recovery from the isolation in RPMI 1640 medium (10% fetal calf serum, 11 mM glucose). Insulin secretion was measured with an islet perifusion system, as described previously elsewhere (Herrington et al., 2006; Tan et al., 2008).

Kv2.2 Silencing in Mouse Islets. A recombinant adenovirus containing a small-hairpin RNA (shRNA) sequence specific to mouse Kv2.2 (Ad-shKv2.2, sequence information available upon request) was constructed by described methods previously elsewhere (Bain et al., 2004). Mouse islets were treated with 4.15 × 10^8 pfu Ad-shKv2.2 48 hours before the static insulin and somatostatin secretion assays and the TaqMan analysis of Kv2.2 mRNA levels.

Intraperitoneal Glucose Tolerance Test. An intraperitoneal glucose tolerance test (IPGTT) was performed with mice (n = 5–7/group) fasted for 5 to 6 hours, as previously

described elsewhere (Tan et al., 2008). GxTX-1E (0.1, 1.0 and 10.0 mg/kg, i.p. injection) or RY796 (50 mg/kg, oral gavage) was administered 30 minutes before the glucose challenge (dextrose 2 g/kg, i.p. injection) with the correspondent vehicle control, which was phosphate-buffered saline for GxTX-1E and 20% vitamin E d-α-tocopheryl polyethyleneglycol succinate for RY796. In the experiments designed to measure plasma hormones, blood samples were harvested via retro-orbital bleeding 20 minutes after glucose injection, followed by insulin, glucagon, or somatostatin measurements by enzyme-linked immunosorbent assay kits (ALPCO Diagnostics, Salem, NH).

Pancreas Perfusion for Insulin and Somatostatin Secretion Measurement. An in situ pancreas perfusion model was used to measure insulin and somatostatin ex vivo (Zhou et al., 1999). Briefly, the aorta and hepatic portal vein were cannulated, and the mouse pancreas was perfused at 1 ml/min with modified Krebs/Ringer buffer. Buffers and the perfusion chamber were saturated with an O$_2$/CO$_2$ gas mixture and were maintained at 37°C. Fractions were collected every minute and assayed for somatostatin by enzyme immunoassay for both SS-14 and SS-28 (Phoenix Peptide, Burlingame, CA) or insulin by radioimmunoassay (Linco Research, St. Charles, MO).

Electrophysiology. Primary islet cells were dispersed, plated on glass coverslips, and cultured for up to 3 days before the patch-clamp studies (Herrington et al., 2006). Kv currents were recorded as described previously elsewhere (Herrington et al., 2005, 2006). The standard pipette solution was (in mM) 120 KCl, 20 KF, 10 EGTA, 10 HEPES, and 2 Mg-ATP, at pH adjusted to 7.2 with KOH. The standard external solution consisted of (in mM) 160 NaCl, 4.5 KCl, 1.8 CaCl$_2$, 1 MgCl$_2$, 10 HEPES, 3 glucose, at pH 7.2 with NaOH. Bovine serum albumin (0.1% wt:vol) was added to solutions containing GxTX-1E.

Statistics. Data are expressed as mean ± S.E. The statistical analysis was conducted using either analysis of variance followed by Newman-Keuls post hoc test using Prism (version 4.0.3; GraphPad Software, La Jolla, CA) or Student's t test, as appropriate. Two-tailed $P < 0.05$ was considered statistically significant.

Results

Genetic Silencing or Pharmacologic Inhibition of Kv2.1 Promotes Insulin Secretion from Pancreatic β-Cells. Previous studies have suggested a role for Kv2.1 in the regulation of GSIS in pancreatic β-cells (MacDonald et al., 2002; Herrington et al., 2006; Jacobson et al., 2007). Consistent with a previous report (Jacobson et al., 2007), whole-cell patch-clamp studies in dispersed islet β-cells revealed ~70% reduction of Kv current in β-cells from Kv2.1$^{-/-}$ mice relative to those from WT littermates (257 ± 18 pA/pF in WT versus 81 ± 7 pA/pF in Kv2.1$^{-/-}$, Fig. 1A). In islet perifusion assays, GSIS elicited by 16 mM glucose was significantly enhanced in Kv2.1$^{-/-}$ islets compared with WT islets (Fig. 1B). In contrast, basal insulin secretion at 2 mM glucose was not altered by Kv2.1 deletion (Fig. 1B), consistent with the idea that Kv2.1 channels are only activated when the β-cell is depolarized and act to limit the amount of calcium that enters the cell to trigger insulin secretion.
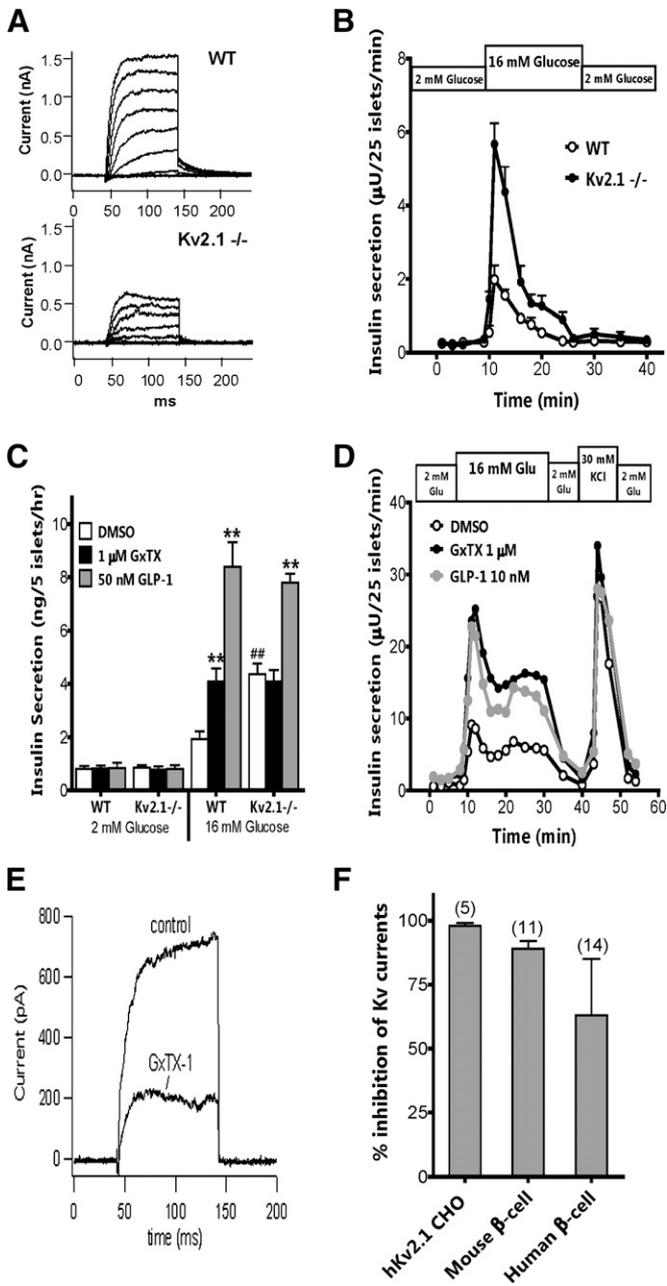
Fig. 1. Kv2.1 channel regulates glucose-stimulated insulin secretion in mouse and human islets. (A) Representative Kv currents in dispersed islet β-cells from WT and Kv2.1$^{-/-}$ mice. Currents were elicited by 100 msec depolarizing steps (in 10 mV increments) from a holding potential of -80 mV. (B) Insulin responses to glucose in islets from WT and Kv2.1$^{-/-}$ mice. Islets were perifused in parallel microchambers with Krebs/Ringer buffer (KRB) medium supplemented with 2 and 16 mM glucose as indicated. Data are mean $\pm$ S.E. of three independent experiments. (C) Basal and glucose-stimulated insulin secretion in isolated WT and Kv2.1$^{-/-}$ islets after exposure to vehicle (DMSO), GxTX-1E (1 μM), or glucagon-like peptide-1 (GLP-1) (50 nM), measured by static incubation assay. Data are mean $\pm$ S.E. of three independent experiments with at least nine replications in total. **$P < 0.01$ versus DMSO at 16 mM glucose in each group; ##$P < 0.01$ versus WT islets after exposure to DMSO at 16 mM glucose; (D) GxTX-1E and GLP-1 enhance GSIS in human islets. Representative perfusion of human islets with KRB medium supplemented with glucose and potassium chloride (KCl) at the indicated concentrations and times. GxTX-1E (1 μM) or GLP-1 (10 nM) was added into the KRB medium during the course of 16 mM glucose perifusion. (E) Representative recording of Kv currents recorded in dispersed human islet β-cells without (control) or with 100 nM GxTX-1E treatment. (F) Percentage inhibition of Kv current by 100 nM GxTX-1E in CHO cells expressing human Kv2.1, and mouse and human islet β-cells. Data on recombinant Kv2.1 and mouse β-cells are taken from Herrington et al. (2006).

GxTX-1E, a 36-amino-acid peptide, selectively inhibits Kv2.1 and Kv2.2 channels with IC$_{50}$ values of 1–3 nM (Herrington et al., 2006) (Supplemental Fig. 1) and augments GSIS in mouse islets (Herrington et al., 2006). However, to date, it has not been determined whether the effect of GxTX-1E in promoting GSIS is mediated through inhibition of Kv2.1, Kv2.2, or both channels. To that end, we first examined the effects of GxTX-1E on GSIS in islets from WT and Kv2.1$^{-/-}$ mice. Addition of GxTX-1E increased insulin secretion at 16 mM glucose by nearly 2-fold in WT islets (Fig. 1C). Compared with WT islets, Kv2.1$^{-/-}$ islets displayed greater insulin secretion in response to 16 mM glucose. However, addition of GxTX-1E did not cause any further increase of GSIS in Kv2.1$^{-/-}$ islets (Fig. 1C). In contrast, in both WT and Kv2.1$^{-/-}$ islets enhancement of GSIS by glucagon-like peptide-1 was indistinguishable, and the basal insulin secretion at 2 mM glucose was not affected by GxTX-1E in either type of islet (Fig. 1C). Thus, the promoting effect of GxTX-1E on mouse islet GSIS appears to be mediated primarily by Kv2.1.

From prior studies (Herrington et al., 2006; Herrington, 2007), it is uncertain whether GxTX-1E or other Kv2.1 inhibitors can promote GSIS in human islets. We thus performed in vitro human islet perifusion assays to assess the effects of GxTX-1E on human islet GSIS. GxTX-1E profoundly enhanced insulin secretion when present in the perfusate during the glucose (16 mM) stimulation phase (Fig. 1D). The average levels of secreted insulin during the 16 mM glucose + GxTX-1E challenge (minutes 10–30) were nearly 3-fold (16.4 $\pm$ 1.5 μU/25 islets/min) greater than those after the challenge with glucose alone (5.8 $\pm$ 0.6 μU/25 islets/min). Once glucose was withdrawn from the perifusion system, secretion was quickly restored to basal levels (Fig. 1D). GxTX-1E did not alter potassium chloride (30 mM) stimulated insulin secretion tested after 10 minutes of washout, indicating that GxTX-1E did not have any prolonged action on either insulin secretion or islet insulin content (Fig. 1D). Whole-cell patch-clamp studies further demonstrated that GxTX-1E significantly suppressed Kv currents in dispersed human β-cells (Fig. 1, E and F). Thus, the selective Kv2.x inhibitor GxTX-1E reduces human β-cell Kv currents and enhances GSIS, similar to its action in mouse β-cells (Fig. 1A and Herrington et al., 2006). Collectively, the data from genetic ablation of Kv2.1 and results with peptidyl Kv2.x inhibitors demonstrate that the Kv2.1 channel is an important target for enhancing GSIS in mouse and human pancreatic β-cells.

Small Molecule Inhibitor of Kv2.1 and Kv2.2 Enhances GSIS in Mouse and Human Islets. To develop additional, nonpeptidyl, pharmacologic tools for Kv2 channels and to explore the chemical tractability of Kv2.1 for small molecule drug development, we screened a fraction of the Merck compound collection with an automated IonWorks electrophysiology assay using a cell line stably expressing human Kv2.1 (Herrington et al., 2011). Two primary hits were found to be selective for Kv2 channels over other Kv channels. Subsequent medicinal chemistry modifications resulted in the optimized small molecule Kv2 inhibitor RY796 (Fig. 2A). RY796 selectively inhibits Kv2.1 and Kv2.2 channels with IC$_{50}$ values of 0.14 and 0.09 μM, respectively, without affecting the activities of a broad range of potassium, sodium, and calcium channels (IC$_{50}$ values > 10 μM) (Herrington et al., 2011). Given the high degree of selectivity, we tested the

A

RY796

B   Mouse islets   **C**   Human islets
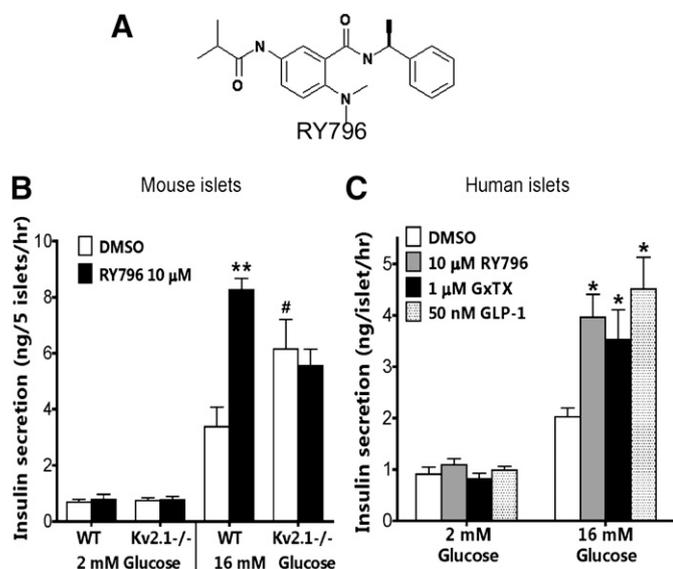
Fig. 2. Small molecule Kv2 inhibitor promotes GSIS in cultured mouse and human islets. (A) Molecular structure of the selective Kv2 inhibitor, RY796. (B) Basal and glucose-stimulated insulin secretion in cultured WT and Kv2.1$^{-/-}$ islets after exposure to vehicle (DMSO) or RY796 (10 μM), measured by static incubation assay. Data are mean $\pm$ S.E. of three independent experiments with nine replications in total. **$P < 0.01$ versus DMSO (vehicle) at 16 mM glucose in each group; #$P < 0.05$ versus WT islets after exposure to DMSO at 16 mM glucose. (C) Basal and glucose-stimulated insulin secretion in human islets after exposure to the indicated agents measured by static incubation assay. Data are mean $\pm$ S.E. of two independent human islet donors with at least six replications in total. *$P < 0.05$ versus DMSO (vehicle).

effects of RY796 on GSIS in isolated mouse and human islets. Similar to GxTX-1E, RY796 significantly enhanced insulin secretion at 16 mM glucose in isolated islets from WT mice and humans (Fig. 2, B and C). In addition, like GxTX-1E, RY796 did not alter GSIS in Kv2.1$^{-/-}$ islets (Fig. 2B). Thus, RY796 shares a similar mechanism to GxTX-1E in enhancing GSIS through inhibition of Kv2.1 channels in islet β-cells.

Kv2.x Inhibitors Do Not Reduce Blood Glucose Levels in Normal Mice. Given their robust stimulatory effects on GSIS in cultured islets, we expected that GxTX-1E and RY796 would have strong in vivo insulinotropic effects and lower systemic blood glucose levels. Lean WT mice were dosed with GxTX-1E (by i.p. injection) or RY796 (by oral gavage) 20 to 30 minutes before an IPGTT. Unexpectedly, GxTX-1E (dosed up to 10 mg/kg) had no significant effect on blood glucose during IPGTT in mice (Fig. 3A). Furthermore, the small molecule Kv2.x inhibitor (RY796) increased, rather than decreased, glucose levels of the IPGTT in lean mice relative to vehicle (20% vitamin E d-a-tocopheryl polyethylene glycol succinate) treated mice (Fig. 3B). By contrast, the small molecule G protein-coupled receptor 40 agonist (Cpd-C, in Tan et al., 2008), a positive control for the study, significantly suppressed the glucose excursion during IPGTT (Fig. 3B). Thus, both Kv2.x inhibitors, GxTX-1E and RY796, failed to display blood glucose-lowering effects in mice in vivo.

The lack of glucose-lowering effect with GxTX-1E or RY796 did not result from insufficient pharmacokinetic exposure of the animals to the inhibitors. The plasma concentrations of GxTX-1E measured at the 60-minute time point of the IPGTT were 2.5 μM in mice treated with 10 mg/kg GxTX-1E. This concentration of GxTX-1E is 800-fold above its IC$_{50}$ value for

inhibition of the cloned Kv2.1 channel (Herrington et al., 2006). The plasma level of RY796 (50 mg/kg) was 12.8 μM, which is 90-fold above its IC$_{50}$ for Kv2.1 (Herrington et al., 2011). Serum protein binding of the agents, which is low for GxTX-1E (Ratliff et al., 2008) and moderate for RY796 (20-fold shift in 33% mouse serum), does not appear to be responsible for the lack of glucose-lowering efficacy. Given these results, we decided to investigate the reason(s) why Kv2.1 inhibitors failed to reduce glucose levels in vivo despite their dramatic enhancement of insulin release in vitro.

Kv2.x Inhibitors Augment Circulating Levels of Somatostatin In Vivo. To uncover these unknown mechanisms, we first examined the circulating levels of islet hormones, including insulin, glucagon, and somatostatin, in mice treated with a Kv2.x inhibitor, followed by a glucose challenge. WT and Kv2.1$^{-/-}$ mice were treated with GxTX-1E (10 mg/kg) 30 minutes before IPGTT, and blood samples were collected 20 minutes after glucose administration, followed by measurements of plasma insulin, glucagon, and somatostatin (SS-14/28) levels. Circulating plasma insulin and glucagon levels were not significantly altered by treatment with GxTX-1E (Fig. 3, C and D). Similar to the experiment in Fig. 3A, basal blood glucose levels in mice treated with GxTX-1E were not different compared with vehicle controls (unpublished data). However, levels of plasma somatostatin (SS-14/28) were significantly elevated in both WT and Kv2.1$^{-/-}$ mice treated with GxTX-1E (Fig. 3E). Similarly, in vivo administration of 50 mg/kg RY796 significantly augmented plasma SS-14/28 levels (190.6 $\pm$ 10.1 pg/ml for RY796 versus 137.8 $\pm$ 3.6 pg/ml for vehicle, $P < 0.05$, $n = 7$), but not insulin (1.92 $\pm$ 0.35 ng/ml for RY796 versus 2.05 $\pm$ 0.53 ng/ml for vehicle, $n = 7$), or glucagon levels (98.5 $\pm$ 5.1 pg/ml for RY796 versus 95.8 $\pm$ 5.8 pg/ml in vehicle, $n = 7$). Thus, in vivo administration of Kv2.x inhibitors leads to an increase in circulating somatostatin.

Kv2.2 Channels Regulate Somatostatin Secretion from Isolated Islets. Pancreatic δ-cells of both rodent and human islets express similar ion channels and secretory machinery as β-cells (Göpel et al., 2000; Zhang et al., 2007; Braun et al., 2009). Importantly, pancreatic δ-cells are known to express Kv2.2 channels (Yan et al., 2004; Wolf-Goldberg et al., 2006). Because GxTX-1E (Herrington et al., 2006) and RY796 (Herrington et al., 2011) inhibit Kv2.2 channels with similar potency as Kv2.1 (Kv2.2 IC$_{50}$ values of 3 nM and 90 nM, respectively), these inhibitors could be affecting δ-cells and β-cells concurrently in pancreatic islets. Thus, we directly measured glucose-stimulated somatostatin release from WT and Kv2.1$^{-/-}$ islets after exposure to these Kv2.x inhibitors. Compared with islets treated with dimethylsulfoxide (DMSO), both inhibitors significantly augmented glucose-stimulated somatostatin secretion in WT and Kv2.1$^{-/-}$ islets (Fig. 4A). Basal somatostatin secretion or glucagon secretion was not altered in the presence of either of these Kv2.x inhibitors (Fig. 4, A and B). Moreover, the stimulatory effects of GxTX-1E or RY796 on somatostatin release in WT and Kv2.1$^{-/-}$ islets were indistinguishable (Fig. 4A), suggesting that Kv2.1 is not involved in the enhanced somatostatin secretion observed in the presence of these Kv2.x inhibitors. These data support the notion that Kv2.2 channels play an important role in regulating somatostatin release from pancreatic δ-cells.

To test this possibility more specifically, we developed an adenoviral vector (Ad-shKv2.2) that carries a small-hairpin
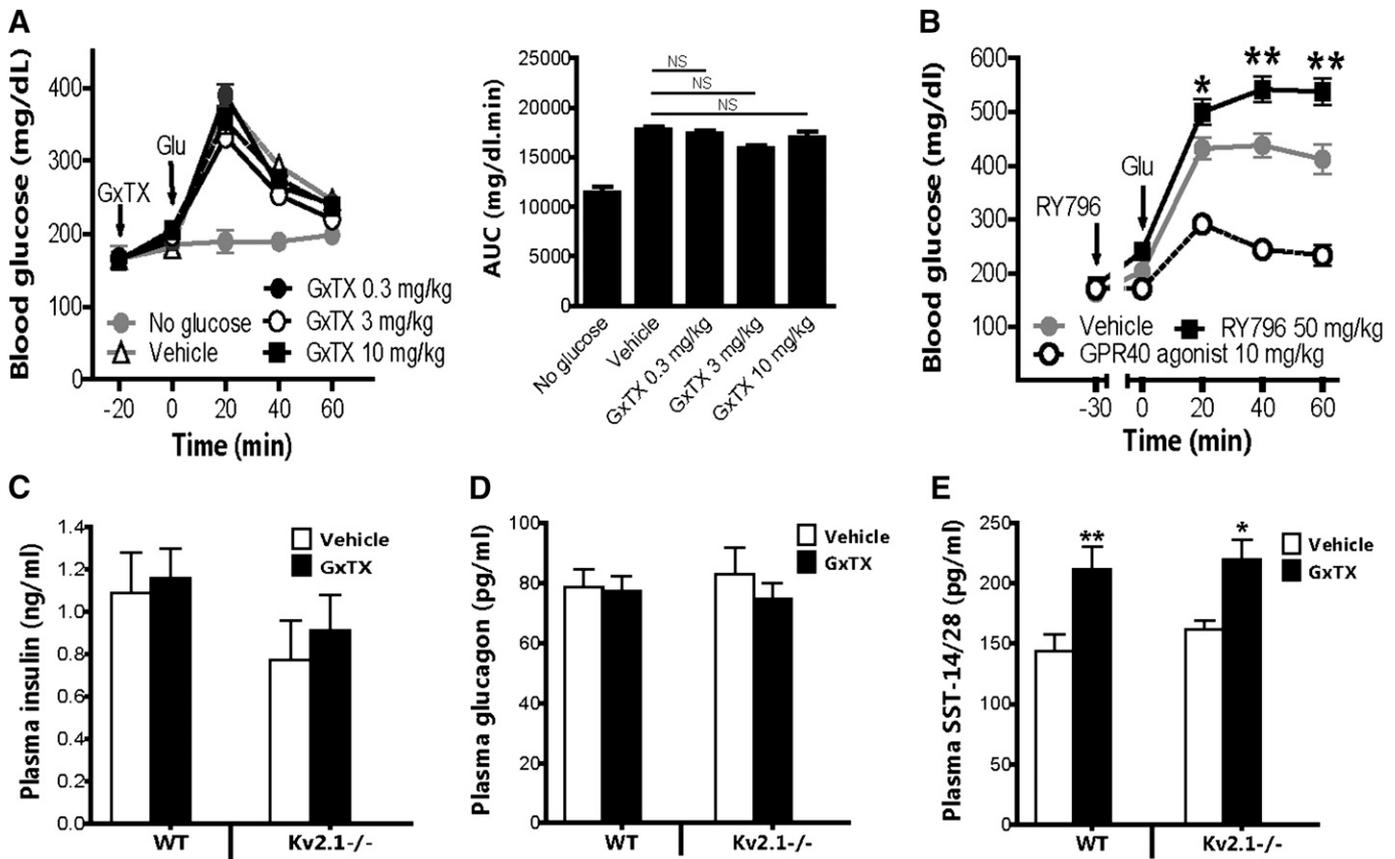
Fig. 3. Kv2 inhibitors fail to limit the glucose excursion in C57BL/6 mice during IPGTT. (A) IPGTT glucose profiles in mice treated with GxTX-1E (i.p. injection 20 minutes before glucose challenge). The area under the curve (AUC) of each group is shown on the right. (B) IPGTT glucose profiles in mice treated with indicated doses of RY796 or G protein-coupled receptor 40 (GPR40) agonist (oral gavage applied 30 minutes before glucose challenge). Data are mean ± S.E. of 7–10 mice per group. *$P < 0.05$; **$P < 0.01$ versus vehicle group. (C–E) Plasma hormone levels 20 minutes after glucose challenge in WT and Kv2.1$^{-/-}$ mice without (vehicle) or with GxTX-1E treatment before glucose challenge: insulin (C) glucagon (D), and somatostatin (E).

RNA specific for mouse Kv2.2 aimed to inhibit expression of Kv2.2 channels in intact isolated islets. The peripheral localization of the δ-cells in mouse islets should favor efficient delivery of the virus to these cells. Transduction of isolated mouse islets with Ad-shKv2.2 for 48 hours led to a ~65% reduction of Kv2.2 mRNA levels (Fig. 4C) and a significant enhancement of glucose-stimulated somatostatin release (Fig. 4D) with no effect on Kv2.1 mRNA expression (data not shown) or islet insulin secretion (Fig. 4E). Likewise, GxTX-1E also significantly enhanced glucose stimulated somatostatin and insulin secretion, but not glucagon release from cultured human islets (Fig. 5, A–C). Taken together, our data suggest that Kv2.1 inhibition augments insulin secretion whereas Kv2.2 inhibition enhances somatostatin release from isolated pancreatic islets studied in vitro.

Intact Pancreas Architecture and Circulation Is Required for Paracrine Inhibition of Insulin Secretion by Somatostatin. Somatostatin is known to inhibit islet β-cell insulin secretion (Mazziotti et al., 2009). However, in cultured islets, treatment with Kv2.x inhibitors elevates β-cell GSIS despite a concomitant elevation in somatostatin secretion. This paradox could be due to a disruption of the normal islet architecture and circulation in isolated pancreatic islets, leading to an impaired paracrine regulation within the islet. We therefore performed in situ pancreas perfusion assays, an ex vivo system where the physiologic δ-cell mass

and native arterial vascular system are preserved. In contrast to what was observed in isolated islets, GxTX-1E did not promote insulin secretion after 16 mM glucose stimulation from either perfused WT or Kv2.1$^{-/-}$ pancreata (Fig. 6, A and B). Kv2.1$^{-/-}$ pancreata did not manifest significant enhancement of insulin secretion at 16 mM glucose (Fig. 6, A and B) compared with WT pancreata, possibly due to their reduced overall pancreas insulin content (Supplemental Fig. 2D). GxTX-1E augmented somatostatin release after 16 mM glucose stimulation from both WT and Kv2.1$^{-/-}$ pancreata (Fig. 6, C and D). The perfused pancreas data are consistent with the in vivo data with Kv2.x inhibitors (Fig. 3), where somatostatin, but not insulin, is elevated. Collectively, our data imply that augmentation of somatostatin release via Kv2.2 inhibition has a powerful inhibitory action on the β-cells that may counteract the stimulatory effects of inhibiting Kv2.1 channels in β-cells.

Blocking Somatostatin Signaling Unmasks Kv2.x Inhibitor Glucose Lowering. Based on the findings in Fig. 6, we reasoned that blocking somatostatin signaling within the islet should lead to net enhancement of insulin secretion in the presence of Kv2.x channel inhibitors. Prior studies (Wang et al., 2005; Sprecher et al., 2010) have suggested that somatostatin suppresses insulin secretion mainly through binding to somatostatin receptor subtype 5 (Sstr5) expressed in pancreatic β-cells. Therefore, we studied
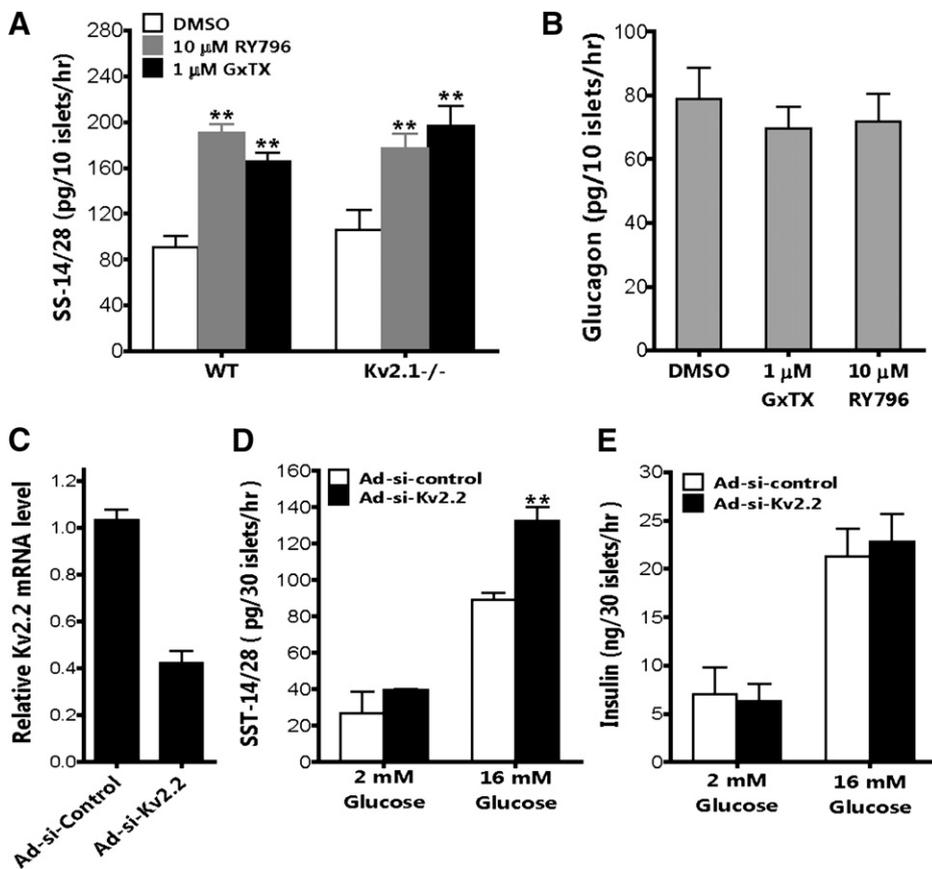
Fig. 4. Effects of inhibition of Kv2.1/Kv2.2 channels on somatostatin release from mouse islets. (A) Levels of somatostatin released from WT and Kv2.1$^{-/-}$ islets exposed to 16 mM glucose plus DMSO, or 1 μM GxTX-1E, or 10 μM RY796 for 60 minutes. (B) Levels of glucagon released from WT mouse islets exposed to 16 mM glucose plus DMSO, or 1 μM GxTX-1E, or 10 μM RY796 for 60 minutes. (A–B) Assays were performed in static incubation assay. Data are mean ± S.E. of three independent experiments with nine replications in total. **$P < 0.01$ compared with vehicle controls in each case. (C) Relative mRNA levels of Kv2.2 in mouse islets 3 days after infection of sh-Kv2.2 or sh-control adenoviruses. (D-E) Basal and glucose-stimulated somatostatin (D) and insulin (E) release from mouse islets 3 days after infection with sh-Kv2.2 or sh-control adenoviruses. (C–E) Data are mean ± S.E. of three independent experiments with nine replications in total. **$P < 0.01$ compared with sh-control for each group.

SSTR5$^{-/-}$ mice lacking *Sstr5*. In perfused SSTR5$^{-/-}$ pancreata, GxTX-1E treatment dramatically increased GSIS (Fig. 7A). Furthermore, GxTX-1E significantly suppressed glucose excursion after glucose challenge in SSTR5$^{-/-}$ relative to SSTR5$^{-/-}$ mice treated with vehicle only (Fig. 7B), and augmented plasma insulin response to glucose challenge

significantly in GxTX-1E-treated SSTR5$^{-/-}$ mice compared with SSTR5$^{-/-}$ mice treated with vehicle (Fig. 7C). Collectively, our data with GxTX-1E suggests that the positive effect of Kv2.1 inhibition in β-cells is likely canceled, at least in part, by stimulation of somatostatin secretion, due to inhibition of Kv2.2 channels in δ-cells.
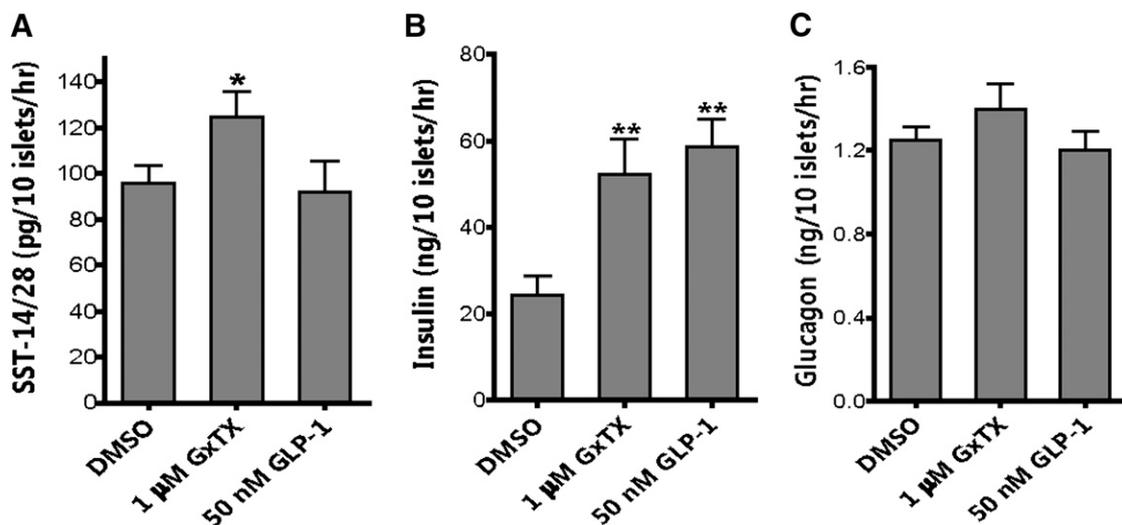
Fig. 5. Kv2 inhibitor GxTX-1E enhances somatostatin and insulin release from cultured human islets. Levels of somatostatin (A), insulin (B), and glucagon (C) released from cultured human islets exposed to 16 mM glucose plus DMSO, or 1 μM GxTX-1E, or 50 nM GLP-1 for 60 minutes were measured by static incubation assay. Data are mean ± S.E. of independent human islet donors with at least six replications in total. *$P < 0.05$ versus DMSO (vehicle).
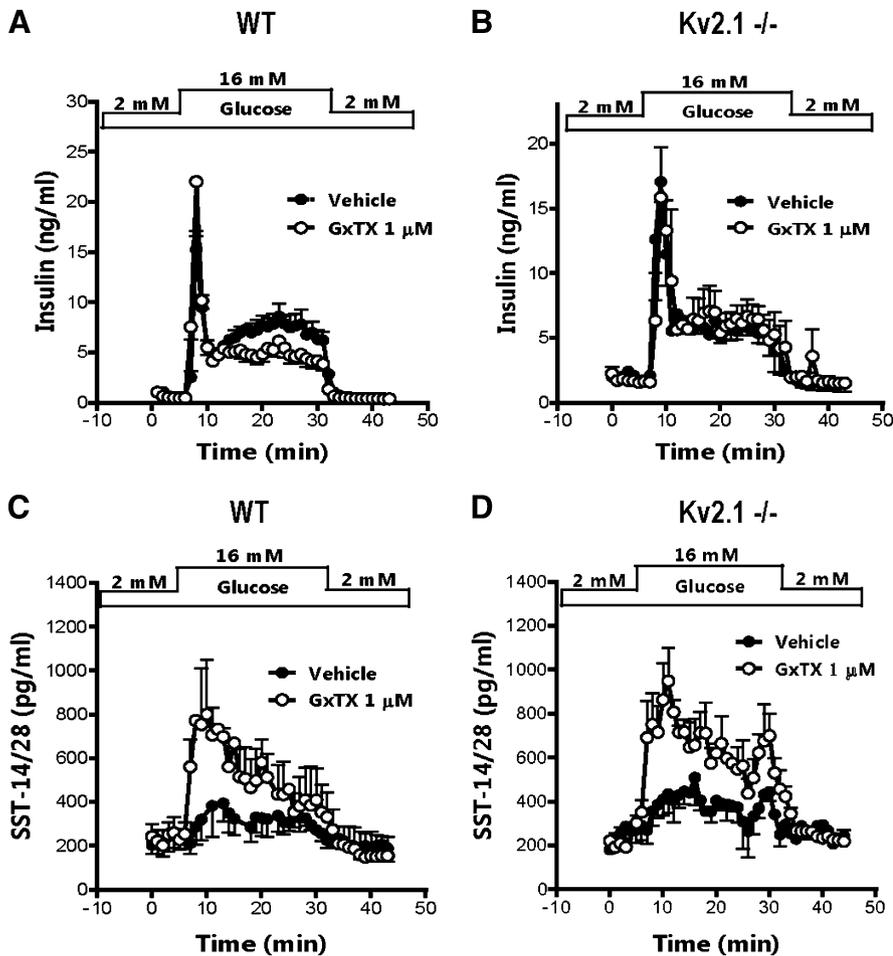
Fig. 6. Effects of GxTX-1E on insulin and somatostatin secretion in perfused pancreata from WT and Kv2.1$^{-/-}$ mice. Release of insulin (A–B) and somatostatin (C–D) in perifused pancreata from WT (A and C) or Kv2.1$^{-/-}$ (B and D) mice. Pancreata were perifused with oxygenated KRB medium supplemented with glucose, at the indicated concentrations and time course, via cannulae placed into the aorta (influx) and portal vein (efflux) in a humidized chamber at 37°C. Fractions of the perfusate were collected once a minute for insulin measurement. GxTX-1E (1 μM) was added only during the 30 minutes when glucose in the KRB was raised to 16 mM. Data are mean ± S.E. of three pancreata for each group.

Discussion

Peptidyl inhibitors of Kv2 channels, such as GxTX-1E, have provided strong evidence that these channels mediate the major voltage-gated K current in rodent pancreatic β-cells, and that their inhibition modulates GSIS (Herrington et al., 2006; Herrington, 2007). In this study, we present pharmacologic evidence that Kv2 channels play a similar role in the regulation of insulin secretion from human β-cells (Figs. 1 and 2). In addition, the role of Kv2.1 and Kv2.2 channels in pancreatic islet hormone release has been elucidated through the use of both genetic and selective peptidyl small molecule Kv2.x inhibitors. All data, taken together, support the idea that the effects of Kv2.x inhibitors on GSIS are mediated primarily by Kv2.1, as these agents have no effect on GSIS in islets lacking the Kv2.1 channel. The present data also suggest that the ability of the Kv2 inhibitors to enhance somatostatin secretion is due to inhibition of Kv2.2 channels present in δ-cells.

Despite the robust effects of Kv2.x inhibitors on islet GSIS in vitro, in vivo administration of GxTX-1E or RY796 did not improve glucose tolerance in the mouse. This lack of correlation between enhanced GSIS in isolated islets and glucose-lowering efficacy in vivo prompted the study of these agents in the perfused pancreas model. The perfused pancreas model preserves the native islet mass and architecture and keeps the arterial vasculature of the pancreas intact. The present data suggest that the perfused pancreas model is a better

predictor of in vivo GSIS efficacy than isolated islet GSIS. For example, inhibition of Kv2.1 channels led to an enhancement of GSIS in isolated islets, but this enhancement did not translate to an increase in insulin secretion from perfused pancreas or to improved glucose tolerance in vivo. One possible explanation for the divergent effects of Kv2 channel inhibition on GSIS in isolated islets versus perfused pancreas or in vivo is a difference in the paracrine regulation of the β-cells.

To explore possible paracrine interactions involved in the regulation of insulin secretion, the other major islet hormones (glucagon and somatostatin) were studied. No changes in glucagon were detected. To our surprise, a significant elevation of plasma somatostatin after glucose challenge in vivo and in situ (perfused pancreas) after GxTX-1E or RY796 treatment was observed. Because Kv2.2 is expressed in pancreatic δ-cells (Yan et al., 2004; Wolf-Goldberg et al., 2006), a role for Kv2.2 in regulating δ-cell somatostatin release was explored. We provide three lines of evidence that Kv2.2, but not Kv2.1, plays a major role in the regulation of δ-cell somatostatin secretion. First, ablation of Kv2.1 had no discernible consequences on somatostatin release in vitro or in vivo. Specifically, basal and glucose-stimulated somatostatin secretion during IPGTT (Fig. 3E), in perfused pancreas (Fig. 4, C and D), or in isolated islets (Fig. 5A) was not different between Kv2.1$^{-/-}$ and WT mice. Second, shRNA-mediated partial knockdown of Kv2.2 expression clearly enhanced somatostatin release in isolated mouse islets. Third, both peptidyl (GxTX-1E) and small molecule (RY796)
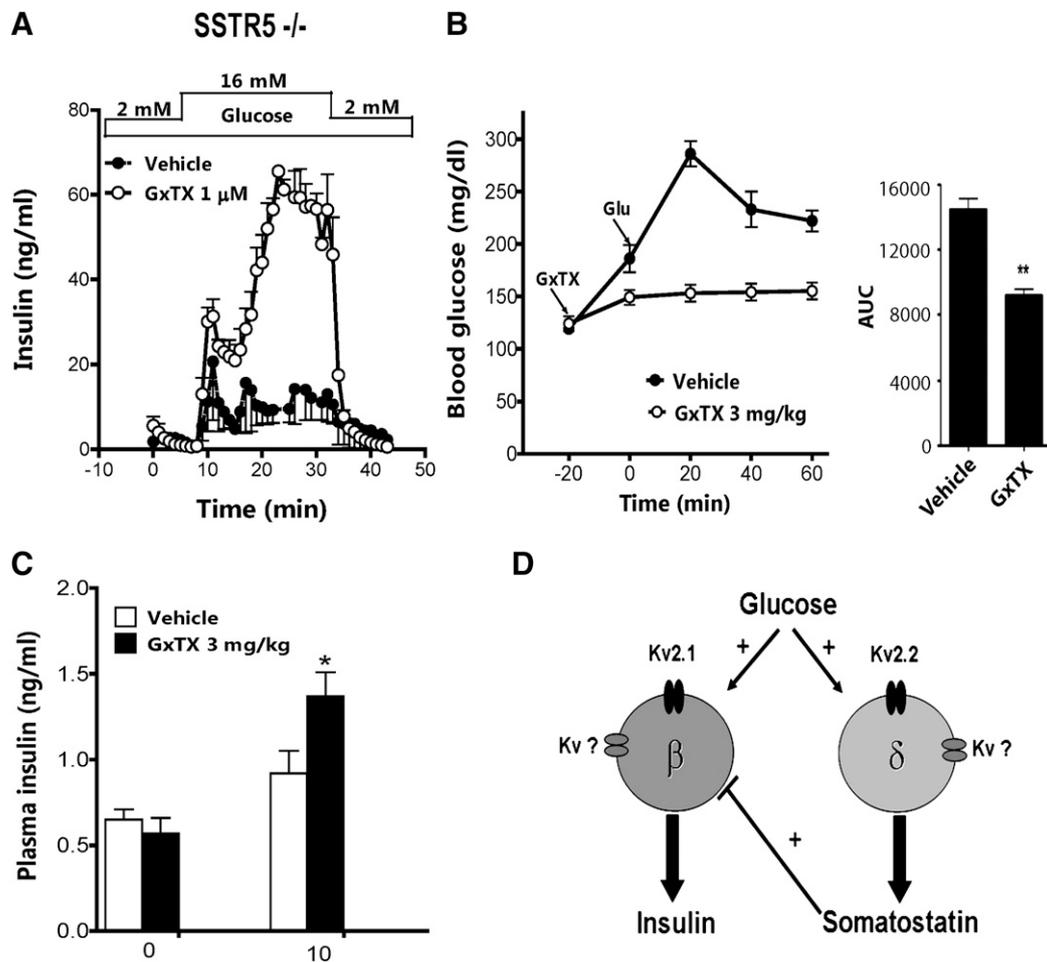
Fig. 7. GxTX-1E stimulates insulin secretion and lowers blood glucose in SSTR5$^{-/-}$ mice. (A) Release of insulin in perifused pancreata from SSTR5$^{-/-}$ mice. Pancreata were perifused with KRB medium supplemented with glucose, at the indicated concentrations and times. GxTX-1E (1 μM) or vehicle was added during the 30 minutes when glucose in the KRB was raised to 16 mM. Data are mean ± SE of three pancreata for each group. (B) Blood glucose levels during an IPGTT in SSTR5$^{-/-}$ mice treated with vehicle or GxTX-1E (3 mg/kg, i.p.) 30 minutes before glucose challenge (dextrose 2 g/kg body weight, i.p.). The area under the curve (AUC) of these two groups is shown on the right. (C) Plasma insulin levels in SSTR5$^{-/-}$ mice during IPGTT before and 10 minutes after glucose challenge. Data are mean ± S.E., n = 6–8 for each group. *P < 0.01 compared with vehicle-treated animals. (D) Illustration showing distinct roles of Kv2.1 and Kv2.2 in pancreatic islets. Kv2.1 primarily regulates GSIS from β-cells, whereas Kv2.2 regulates somatostatin release from δ-cells.

Kv2.x inhibitors robustly enhanced glucose stimulated somatostatin secretion, even in the absence of Kv2.1.

Our understanding of the biology of the pancreatic δ-cells is limited, largely due to the difficulty of isolating these cells (Göpel et al., 2000; Zhang et al., 2007). Expression of Kv2.2 protein in δ-cells has been reported (Yan et al., 2004; Wolf-Goldberg et al., 2006), and δ-cells indeed have Kv currents (Göpel et al., 2000; Braun et al., 2009). However, the impact of inhibiting Kv2.2 on δ-cell physiology and somatostatin release had not been investigated in detail until the present study. Braun et al. (2009) detected delayed rectifying Kv2 current in purified δ-cells, yet blocking the current with tetraethylammonium or stromatoxin did not affect somatostatin release in intact human islets. In contrast, we find that Kv2 inhibition by GxTX-1E enhances somatostatin release in human islets (Fig. 5). The reason(s) for this discrepancy is not clear but may be explained by differences in the pharmacologic agents used. Tetraethylammonium is a nonselective Kv channel blocker and inhibits other classes of channels as well. Also, although GxTX-1E is highly selective against other ion channels present in β-cells, the selectivity of stromatoxin for these channels, in

particular voltage-gated calcium channels, is not well defined. Further studies will be required to definitively address the role of Kv2.2 in insulin and somatostatin secretion.

The prominent role of Kv2.2 in the regulation of somatostatin release raises several questions for future studies. For example, is the lack of paracrine somatostatin action detected in the isolated islet GSIS assay due to loss of δ-cells from the periphery of the islet during the islet isolation procedure? Also, is the lack of in vivo glucose lowering seen with pan-Kv2 inhibitors due solely to paracrine somatostatin? The data on SSTR5$^{-/-}$ mice suggest somatostatin is the major factor preventing the in vivo efficacy of pan-Kv2 inhibitors. Because inhibition of Kv2.2 channels augmented somatostatin secretion from isolated islets and perfused pancreata, the major source of somatostatin is likely to be the δ-cells within the islet. However, our data do not exclude the possibility that systemically administered pan-Kv2 inhibitors may also cause release of somatostatin from another source, such as the gastrointestinal mucosa. Compared with the untreated cohorts, GxTX-1E significantly lowered the basal glucose level in SSTR5$^{-/-}$ mice, (Fig. 7B), suggesting that the inhibition of

Kv2 channels may have additional effects on peripheral tissues other than the pancreas. Our data also cannot exclude the possibility that pan-Kv2 inhibitors regulate Kv2.2 in cell types other than δ-cells in the pancreas.

Recently, Kv2.1 null (Kv2.1$^{-/-}$) mice have been reported to have reduced fasting blood glucose levels and elevated insulin secretion in vivo (Jacobson et al., 2007). In contrast, we did not observe any clear differences between Kv2.1$^{-/-}$ mice and their WT littermate controls in plasma glucose and insulin levels during an IPGTT (Supplemental Fig. 2). The Kv2.1 knockout mice used by Jacobson et al. (2007) and by us were rederived from the same original line generated at Deltagen. The exact reason for the discrepancy between the glycemic phenotypes of the two sublines of Kv2.1$^{-/-}$ mice is not known. Our Kv2.1$^{-/-}$ mice weighed significantly less than their WT littermates and displayed improved peripheral insulin sensitivity (Supplemental Fig. 2). The insulin content of the Kv2.1$^{-/-}$ mouse pancreas was significantly reduced compared with WT littermate control (Supplemental Fig. 2D), suggesting reduced β-cell mass in this model. Given the marked divergence in body weight, insulin sensitivity, and pancreatic insulin content between the WT and Kv2.1$^{-/-}$ mice, the utility of the Kv2.1$^{-/-}$ mouse model to discern the role played by Kv2.1 in whole body glucose metabolism is uncertain.

In summary, the results of the present study are consistent with a simple model where two closely related members of the Kv2 family play distinct roles in regulating pancreatic islet hormone release (Fig. 7D) in mice. In this model, Kv2.1 channels in the β-cell regulate GSIS, whereas in the δ-cell, Kv2.2 channels function to control the release of somatostatin. It is important to note that this model does not exclude the possibility that other Kv channels may also participate in the regulation of insulin secretion from the β-cell. Although the precise mechanism by which somatostatin attenuates insulin secretion has not been explored in the present study, we speculate that somatostatin, by binding to specific receptors in β-cells, activates an inward rectifier potassium conductance that hyperpolarizes β-cells (Smith et al., 2001) and counteracts the action potential prolongation caused by Kv2.1 inhibition. Given that the interconnection between somatostatin and insulin release has been studied extensively in mice, it would be important to understand if somatostatin tone would also be a factor in limiting GSIS in humans when using a nonselective Kv2.x channel inhibitor. Given the importance of islet hormones in glucose homeostasis and the excellent track record of insulin secretagogues for successful blood glucose control in the clinic (Doyle and Egan 2003), development of a selective Kv2.1 inhibitor may provide a new avenue for the treatment of type 2 diabetes.

Author Contributions

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Conducted experiments: Li, Herrington, Petrov, Ge, Jensen, Hohmeier, Wagner.

Contributed new reagents or analytic tools: Xiong.

Performed data analysis: Li, Herrington, Petrov, Ge, Jensen, Hohmeier, Wagner, Zhou.

Wrote or contributed to the writing of the manuscript: Li, Herrington, Newgard, Garcia, Kaczorowski, Zhou.

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