Research Article | Islet Studies

A Glycine-Insulin Autocrine Feedback Loop Enhances Insulin Secretion From Human β-Cells and Is Impaired in Type 2 Diabetes

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Abstract

The secretion of insulin from pancreatic islet β-cells is critical for glucose homeostasis. Disrupted insulin secretion underlies almost all forms of diabetes, including the most common form, type 2 diabetes (T2D). The control of insulin secretion is complex and affected by circulating nutrients, neuronal inputs, and local signaling. In the current study, we examined the contribution of glycine, an amino acid and neurotransmitter that activates ligand-gated Cl^- currents, to insulin secretion from islets of human donors with and without T2D. We find that human islet β-cells express glycine receptors (GlyR), notably the GlyRα1 subunit, and the glycine transporter (GlyT) isoforms GlyT1 and GlyT2. β-Cells exhibit significant glycine-induced Cl^- currents that promote membrane depolarization, Ca^{2+} entry, and insulin secretion from β-cells from donors without T2D. However, GlyRα1 expression and glycine-induced currents are reduced in β-cells from donors with T2D. Glycine is actively cleared by the GlyT expressed within β-cells, which store and release glycine that acts in an autocrine manner. Finally, a significant positive relationship exists between insulin and GlyR, because insulin enhances the glycine-activated current in a phosphoinositide 3-kinase–dependent manner, a positive feedback loop that we find is completely lost in β-cells from donors with T2D.

Many neurotransmitters modulate insulin secretion by changing the electrical activity of β-cells via ion channels and receptors (1–4). These signals may originate from the circulation, neuronal fibers, and/or pancreatic islets (5). Proper neurotransmitter signaling is critical for coordinating insulin secretion from all the islets in the pancreas, and dysfunction in this signaling may be involved with the pathogenesis of diabetes. A multitude of recent metabolic studies investigating biomarkers for type 2 diabetes (T2D) have identified glycine as a potential candidate (6–16). A strong correlation exists between plasma glycine concentrations and insulin sensitivity (7,13), glucose disposal (8), and obesity (9,17). Circulating plasma glycine concentrations are inversely related to the risk of T2D (6–8). Furthermore, glycine supplementation raises plasma insulin (18,19).

Glycine is a nonessential amino acid found abundantly in collagen-rich foods. In the central nervous system, glycine acts as an inhibitory neurotransmitter by activating a family of ligand-gated Cl^- channels called glycine receptors (GlyR). These belong to the pentameric ligand-gated ion channel family and are composed of two α- and three β-subunits (20). In addition to glycine, GlyR are also activated by β-alanine and taurine, whereas strychnine is a potent and specific inhibitor (20). Extracellular glycine concentrations are regulated by glycine transporters (GlyT), which belong to the Na^+-dependent solute carrier family 6 (SLC6) transporters, where glycine transporter 1 (GlyT1) cotransports two Na^+, one Cl^-, and one glycine, and glycine transporter 2 (GlyT2) cotransports three Na^+, one Cl^-, and one glycine (21).

Here we have investigated the role for glycine in insulin secretion from human pancreatic islets of Langerhans. We demonstrate that human β-cells express GlyR, notably GlyRα1, which mediate a depolarizing Cl^- current that enhances action potential firing, Ca^{2+} entry, and insulin secretion. This GlyR-mediated current is enhanced by insulin in β-cells from donors without T2D, but not in β-cells from donors with T2D, where we find GlyRα1 protein expression and glycine-activated currents are downregulated. Furthermore, human β-cells express GlyT1 and GlyT2 that mediate the uptake of glycine, which is released from β-cells by Ca^{2+}-dependent exocytosis. Thus, an autocrine glycine-insulin feedback loop positively regulates insulin secretion, and its dysfunction may contribute to impaired secretion in human T2D.

Research Design and Methods

Cell Culture and Transfection

Islets from 50 human donors were isolated by the Alberta Diabetes Institute IsletCore (22) or the Clinical Islet Laboratory (23) at the University of Alberta, with appropriate ethical approval from the University of Alberta Human Research Ethics Board (Pro00013094; Pro 00001754). Donor information is described in Supplementary Tables 1 and 2. Mouse islets were from male C57/Bl6 mice at 10–12 weeks of age, and experiments were approved by the University of Alberta Animal

onto Petri dishes or coverslips and cultured in RPMI medium (Corning or Life Technologies) containing 7.5 mmol/L glucose for >24 h before the experiments. For measurement of glycine release, the cells were transfected with a plasmid encoding the mouse GlyRα1 subunit (provided by G.E. Yevenes and H.U. Zeilhofer, University of Zurich [24]) using Lipofectamine 2000 (Life Technologies). A GFP-encoding plasmid was included as a transfection marker (pAdtrackCMV).

Electrophysiology

Patch pipettes were pulled from borosilicate class and fire-polished (tip resistance 4–7 MegaΩ for most experiments and 3–4 MegaΩ for Ca^{2+} infusion experiments). Patch-clamp recordings were performed in the standard or perforated-patch whole-cell configurations by using an EPC10 amplifier and Patchmaster software (HEKA Electronik). The cells were continuously perifused with extracellular solution (except during stopped-flow experiments) at a bath temperature of ~32°C. After experiments, the cell types were established by immunocytochemistry, as previously described (25). For membrane potential and current recording, Krebs-Ringer buffer (KRB) composed of (in mmol/L) 140 NaCl, 3.6 KCl, 0.5 $MgSO_4$, 1.5 $CaCl_2$, 10 HEPES, 0.5 NaH_2PO_4, 5 $NaHCO_3$, and 6 glucose (pH adjusted to 7.4 with NaOH) was used as bath solution. For measurements of glycine release, the extracellular medium contained (in mmol/L) 118 NaCl, 20 tetraethylammonium-Cl, 5.6 KCl, 2.6 $CaCl_2$, 1.2 $MgCl_2$, 5 HEPES, and 6 glucose (pH adjusted to 7.4 with NaOH). For perforated-patch experiments, the pipette solution contained (in mmol/L) 76 K_2SO_4, 10 KCl, 10 NaCl, 1 $MgCl_2$, 5 HEPES, and 0.24 mg/mL amphotericin B or 50 µg/mL gramicidin (pH adjusted to 7.35 with KOH). Glycine-evoked membrane currents were recorded with an intracellular solution containing (in mmol/L) 130 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 EGTA, 5 HEPES, and 3 MgATP (pH adjusted to 7.2 with KOH). For glycine release measurements, the pipette solution was composed (in mmol/L) of 120 CsCl, 1 $MgCl_2$, 9 $CaCl_2$, 10 EGTA, 10 HEPES, 3 MgATP, and 0.1 cAMP (pH adjusted to 7.2 with CsOH). The cells were clamped at −70 mV during the recordings. For glycine release experiments, analysis was conducted with Mini Analysis 6 software (Synaptosoft).

Immunohistochemistry

Deparaffinized human pancreatic tissue sections were heated in 10 mmol/L Na^+ citrate (pH 6) for 15 min, followed by a 15-min cooling step in the same buffer. The sections were rinsed in PBS and blocked with 20% goat serum for 30 min. Antibodies against the GlyRα1 subunit (diluted 1:100 in 5% goat serum; Synaptic Systems), GlyRα3 subunit (diluted 1:100 in 5% donkey serum; Santa Cruz Biotechnology), GlyT1 (diluted 1:100 in 5% donkey serum; Santa Cruz Biotechnology), or GlyT2 (diluted 1:200 in 5% goat serum; Atlas Antibodies) were then added to the sections and incubated overnight. Sections were washed with PBS and then antibodies for insulin (diluted 1:1000 in goat serum [Sigma-Aldrich] and diluted 1:100 in 5% donkey serum [Santa Cruz Biotechnology]) were added to the sections and incubated for 60 min. After a washing step in PBS, the sections were incubated with fluorescently labeled secondary antibodies Alexa Fluor

(Life Technologies). Images were captured using an inverted microscope equipped with a Zeiss Colibri imaging system. Quantitative imaging of GlyR in islets from donors with and without T2D at constant exposure times were analyzed with Volocity (PerkinElmer). Background subtractions were performed with ImageJ software (National Institutes of Health, Bethesda, MD).

Measurements of $[Ca^{2+}]_i$

Dispersed islet cells were incubated in culture medium containing 1 μmol/L Fura-2 AM (Life Technologies) for 10 min. The coverslips were mounted onto an inverted microscope and perifused with KRB containing 6 mmol/L glucose (unless otherwise indicated). Fluorescence was excited at 340 and 380 nm (intensity ratio 5:2) using an Oligochrome light source (TILL Photonics) and a ×20 objective (Zeiss Fluar). Emission was monitored at 510 nm, and images were captured at 0.5 Hz using an intensified charge-coupled device camera and Life Acquisition software (TILL Photonics). Cell types were identified after the experiment by immunocytochemistry, as previously described (25). Excitation ratios (340/380 nm) were subsequently calculated offline from captured images in regions of interest corresponding to identified cell types, using ImageJ software.

Insulin Secretion

Insulin secretion was measured in static secretion assays as described previously (26) or by perifusion at 37°C in KRB with (in mmol/L) 115 NaCl, 5 KCl, 24 $NaHCO_3$, 2.5 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 0.1% BSA (pH 7.4 with NaOH). For perifusion, 15 islets per lane were perifused (0.25 mL/min) with 1 mmol/L glucose KRB (with or without 1 μmol/L strychnine) for 24 min and then with the indicated condition. Samples were collected over 2-min intervals. Islets were lysed in acid/ethanol buffer (1.5% concentrated HCl, 23.5% acetic acid, and 75% ethanol) for total insulin content. Samples were assayed using the Insulin Detection Kit (Meso Scale Discovery).

Data Analysis

Data are expressed as means ± SEM unless indicated otherwise. Statistical significance was evaluated using the Student *t* test or two-way ANOVA. *P* values <0.05 were considered significant.

Results

GlyR Expression in Human Islets From Donors With and Without T2D

RT-PCR analysis revealed expression of the GlyR subunits α1, α3, and β in human islets (Supplementary Fig. 1*A* and *B*). Quantitative immunofluorescence was performed in pancreatic tissue sections taken from donors without T2D (4 donors) and donors with T2D (4 donors) to measure GlyRα1 and GlyRα3 protein levels in β-cells. Immunostaining for the GlyRα1 and GlyRα3

donors without T2D (480 ± 35 average pixel intensity [PI], 4 donors) than in donors with T2D (309 ± 24 average PI, 4 donors; $P < 0.001$) (Fig. 1*B* and Supplementary Fig. 1*C*), whereas GlyRα3 expression appeared lower than that of GlyRα1 and was not different between donors without T2D (304 ± 24 average PI, 4 donors) and with T2D (268 ± 30 average PI, 4 donors) (Fig. 1*C* and *D* and Supplementary Fig. 1*D*).

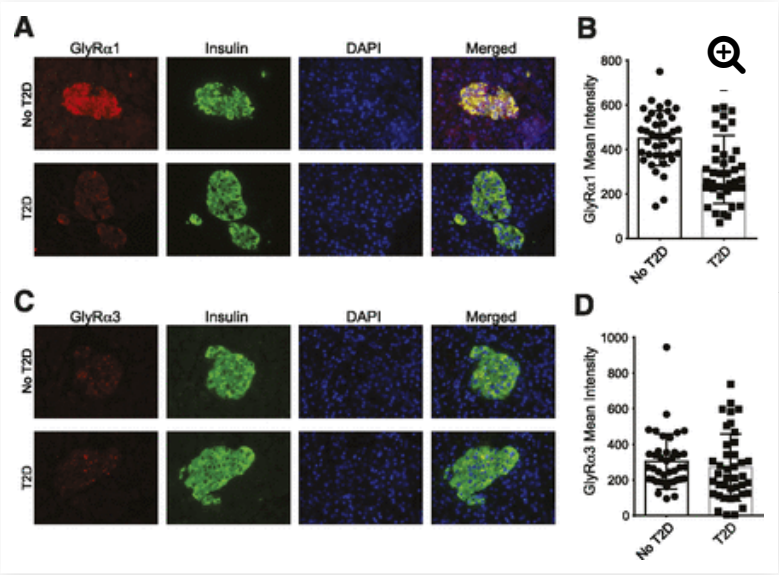

Figure 1

Expression of GlyR in human islets. *A*: Representative images of GlyRα1 expression in pancreatic tissue sections from donors with and without T2D from quantitative immunofluorescence (GlyRα1, red; insulin, green; DAPI, blue). *B*: Average GlyRα1 PI in insulin-positive cells from donors with and without T2D. *C*: Representative images of GlyRα3 expression in pancreatic tissue sections from donors with and without T2D from quantitative immunofluorescence (GlyRα3, red; insulin, green; DAPI, blue). *D*: Average GlyRα3 PI in insulin-positive cells from donors with and without T2D. The scatter plots represent values for individual islets from four donors in each group. ***$P < 0.001$.

Consistent with the detection of GlyR subunits by immunocytochemistry, we observed substantial glycine-activated Cl^- currents in human β-cells, which were positivity identified by insulin immunostaining after the experiment (Fig. 2*A* and *B*). Extracellular application of glycine (300 µmol/L) evoked large inward currents in 56 of 62 human β-cells (Fig. 2*Ai*). The current amplitude averaged −316 pA (−26 ± 3 pA/pF, 9 donors) (Fig. 2*B*) and was half-maximally activated by 90 µmol/L glycine ($n = 9$ cells, 2 donors) (Fig. 2*C*). Glycine-evoked currents were reduced by 92 ± 3% ($n = 7$ cells, 4 donors; $P < 0.001$) in the presence of 1 µmol/L strychnine. Glycine-evoked, strychnine-sensitive currents were also found in α-cells, but the currents were less frequently detected (3 of 10 cells) and the amplitudes were much smaller compared with β-cells (−9.3 ± 2.1 pA and −3.2 ± 0.5 pA/pF, 3 donors) (Fig. 2*Aii*). In mouse β-cells glycine evoked a strychnine-sensitive current in only 3 of 15 β-cells (−26 ± 19 pA and −4.5 ± 2 pA/pF) (Fig. 2*Aiii*), whereas glycine did not evoke a current in rat β-cells ($n = 11$ cells; data not shown), suggesting species-

the reduced GlyRα1 immunostaining in islets from donors with T2D, we find in β-cells from donors with T2D that glycine-evoked currents are significantly smaller than in those from control donors without T2D (−12 ± 2 pA/pF, n = 36 cells, 5 donors; P < 0.001) (Fig. 2*Aiv* and *B*). The glycine-sensitive current demonstrated a reversal potential consistent with that expected of a Cl^--mediated current in our solutions (13.5 mV, n = 18 cells, 4 donors) (Fig. 2*D*).

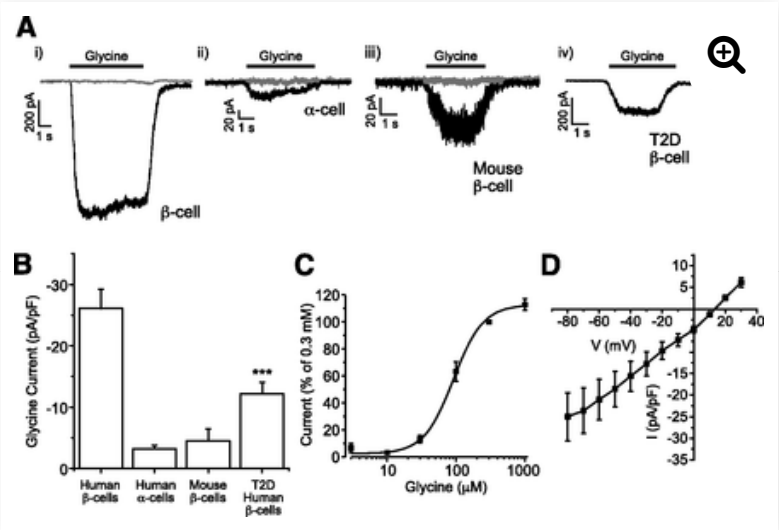

Figure 2 Download figure | Open in new tab | Download powerpoint

Detection of GlyR-mediated Cl^- currents by whole-cell patch-clamping in human islet cells. *A*: Representative traces showing glycine (300 μmol/L)–evoked inward currents (black traces) in β-cells from a donor without T2D (*i*), in α-cells (*ii*), in mouse β-cells (*iii*), and in human β-cells from a donor with T2D (*iv*). Gray traces were obtained in the presence of 1 μmol/L strychnine. Note the different scale bars. *B*: Glycine-evoked inward currents normalized to cell size. *C*: Dose-response curve of glycine-evoked currents in human β-cells. Responses were normalized to those obtained with 300 μmol/L glycine. *D*: Current-voltage relationship of currents evoked by 300 μmol/L glycine. ***P < 0.001 compared with cells from donors without T2D.

We then investigated the effect of glycine on the membrane potential of human β-cells using the perforated-patch configuration. In the presence of 6 mmol/L glucose, extracellular application of glycine (100 μmol/L) depolarized the cells and increased action potential firing (Fig. 3). In one set of experiments (Fig. 3*A–C*), glycine evoked significantly more frequent action potential firing (2.9 ± 0.5 Hz, n = 6 cells, 2 donors) compared with control cells (0.6 ± 0.2 Hz, n = 6 cells, 2 donors; P < 0.01) (Fig. 3*A* and *B*), and decreased action potential height (46 ± 3 mV, n = 6 cells, 2 donors) compared with control cells (56 ± 3 mV, n = 6 cells, 2 donors; P < 0.05) (Fig. 3*A* and *C*). In a separate set of experiments, this effect was prevented by strychnine (Fig. 3*D*). Glycine depolarized human β-cells by an average of 15 ± 3 mV (n = 7, 3 donors; P < 0.01) (Fig. 3*E*).

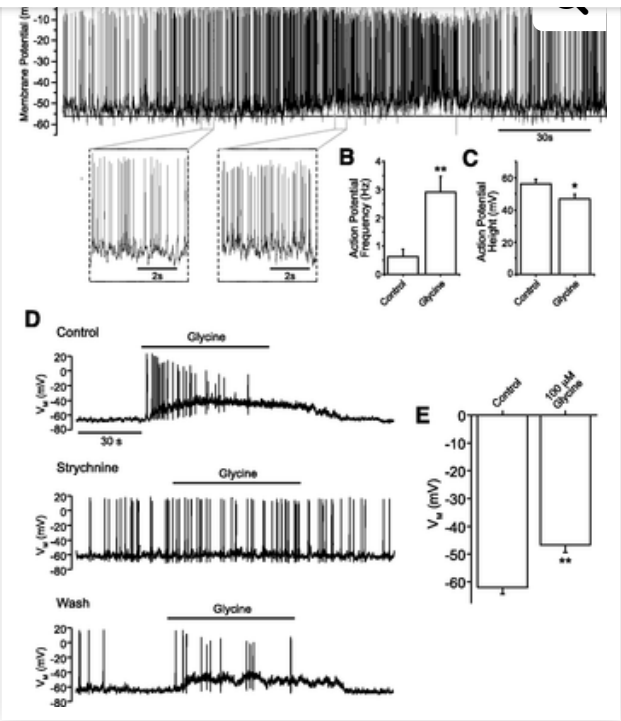

Figure 3 Download figure | Open in new tab | Download powerpoint

Effect of glycine on membrane potential in human β-cells. Membrane potential recordings performed using the perforated-patch configuration. *A*: Sample trace of the effect of glycine (100 µmol/L) on human β-cells. Application of 100 µmol/L glycine elicited a significant increase in action potential firing (*B*) and a reduced action potential height (*C*). *D*: Sample trace of glycine-dependent (100 µmol/L) β-cell depolarization before, during, and after application of 1 µmol/L strychnine. *E*: Change in membrane potential in response to 100 µmol/L glycine. **P* < 0.05 and ***P* < 0.01.

Glycine Modulates $[Ca^{2+}]_i$ in β-Cells

We next examined the effects of glycine on $[Ca^{2+}]_i$ in human islet cells. The identities of all the cells were confirmed after the experiments by immunostaining (Fig. 4*A*). A representative recording from a β-cell is shown in Fig. 4*B*. The application of glycine increased $[Ca^{2+}]_i$ in 50% (72 of 144) of all β-cells tested (7 donors). The responses were similar after addition of 100 µmol/L and 300 µmol/L glycine (55% and 42% responding cells, respectively) and were abolished in the presence of strychnine (Fig. 4*B* and *C*). Interestingly, in 7% (10 of 144) of the β-cells, glycine decreased rather than increased $[Ca^{2+}]_i$, but no clear effect was observed in the remaining 43% of cells (Fig. 4*D*). We compared the baseline $[Ca^{2+}]_i$ (before glycine addition, at 6 mmol/L extracellular glucose) in cells that showed an increase, no change, and a decrease in $[Ca^{2+}]_i$ upon glycine application, respectively (Fig. 4*E*). Cells that responded with a $[Ca^{2+}]_i$ rise had a significantly lower baseline $[Ca^{2+}]_i$ than nonresponders, whereas a significantly higher baseline $[Ca^{2+}]_i$ was observed in cells showing a decrease after glycine administration. At 1 mmol/L glucose, the proportion of cells responding with a $[Ca^{2+}]_i$ increase (73% [11 of 15 cells], 2 donors) was higher than at 6 mmol/L glucose (43% [41 of 97 cells]) and at 10 mmol/L glucose (56% [18 of

glucose (0.86 ± 0.02 AU). A clear increase in $[Ca^{2+}]_i$ upon addition of glycine was sometimes also observed in α-cells (Fig. 4*F*), but the proportion of responding cells was much lower compared with β-cells (11% [14 of 124 cells], 6 donors), consistent with the lower proportion of α-cells that exhibited a glycine-activated Cl^- current. These also tended to require a higher glycine concentration, with 7% of the cells responding to 100 µmol/L and 19% responding to 300 µmol/L. Finally, extracellular Ca^{2+} was necessary to elicit a response (Fig. 4*G*), and the absence of Ca^{2+} suppressed the response to glycine by 99.8% (n = 35 cells, 5 donors; P < 0.01) (Fig. 4*H*). Additional traces and expanded time scales for these experiments are shown in Supplementary Fig. 4.

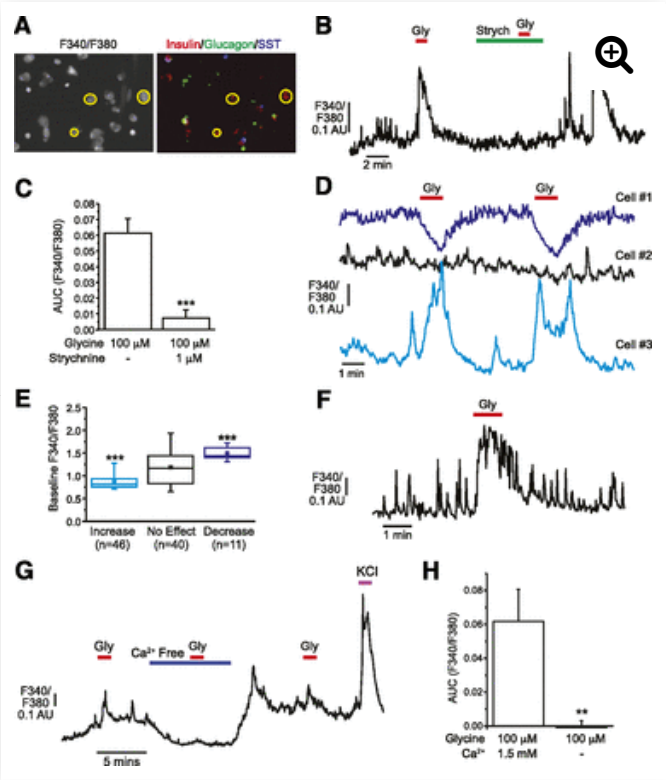

Figure 4

Effect of glycine on $[Ca^{2+}]_i$ in islet cells. *A*: Ratiometric images were recorded in dispersed islet cells, followed by determination of cell types by immunostaining. SST, somatostatin. *B*: Representative trace shows effects of glycine (Gly) (100/100/300 µmol/L) on $[Ca^{2+}]_i$ in a β-cell in the absence or presence of strychnine (Strych) (1 µmol/L). *C*: Area under curve (AUC) during 1-min application of 100 µmol/L glycine in the absence or presence of strychnine (1 µmol/L) in the same cells (n = 39 cells from 9 experiments). Baseline values (before glycine application) were subtracted. *D*: Traces from three β-cells from the same experiment illustrating different types of $[Ca^{2+}]_i$ responses to glycine (100/300 µmol/L). The traces are shown to scale (i.e., without adjustment of baseline values). *E*: Baseline fluorescence ratios (before glycine application) in β-cells that responded with an increase, no change, or a decrease in $[Ca^{2+}]_i$, respectively (n = 38, 40, and 10). Data are shown as box plots (squares, means; lines, medians; boxes, 25th/75th percentiles; whiskers, minimum/maximum values). *F*: Effect of glycine (300 µmol/L) on $[Ca^{2+}]_i$ in an α-cell. *G*: Representative trace shows effects of glycine on $[Ca^{2+}]_i$ in the absence or presence of extracellular Ca^{2+} and the Ca^{2+} response to 70 mmol/L KCl. *H*: AUC during a 1-min application of 100 µmol/L glycine in the absence or presence of extracellular Ca^{2+}. $^{**}P$ < 0.01 and $^{***}P$ < 0.001.

insulin enhances Cl^- currents in neurons (27,28). We investigated whether insulin has a role in enhancing glycine signaling in human islets. In β-cells acutely treated with 10 μmol/L insulin, the glycine-evoked current was amplified by 54 ± 17% (n = 23, 8 donors; $P < 0.01$) compared with control cells (Fig. 5*Ai* and *B*). We confirmed similar results in response to treatment with 100 nmol/L insulin (data not shown). We suspected that this was a result of signaling between the insulin receptor and GlyR, so we preincubated the cells with 100 nmol/L wortmannin, a phosphoinositide 3-kinase inhibitor, to inhibit insulin receptor signaling. Wortmannin prevented the insulin-dependent amplification of glycine-evoked current in donors without T2D (a 6 ± 4% increase, n = 12 cells, 5 donors; not significant) (Fig. 5*Aii* and *B*). Interestingly, β-cells from donors with T2D did not show an amplification of glycine-evoked current in response to insulin (a 5 ± 9% change from baseline, n = 13 cells, 4 donors; not significant) (Fig. 5*Aiii* and *B*).

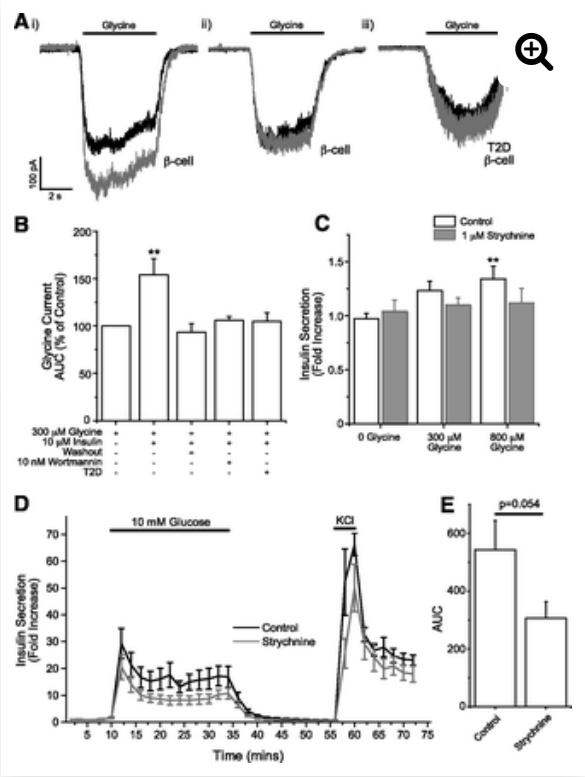

Figure 5

Glycine-insulin interaction. *A*: Representative trace of insulin-dependent amplification of glycine current in human β-cells from donor without T2D (*i*), β-cells preincubated with 100 nmol/L wortmannin for 1 h (*ii*), and β-cells from donors with T2D (*iii*). Control glycine currents before treatment are in black, and glycine currents after treatment with 10 μmol/L insulin for 1 min are in gray. Similar results were obtained with 100 nmol/L insulin (data not shown). *B*: Area under the curve (AUC) normalized to control glycine currents. At 6 mmol/L glucose, 300 μmol/L glycine moderately increased insulin secretion, whereas 800 μmol/L glycine strongly increased insulin secretion. *C*: Glycine induced insulin secretion in intact human islets. Glycine dose-dependently increased insulin secretion in a strychnine-sensitive manner. *D*: Dynamic glucose-stimulated insulin secretion perifusion in control and human islets treated with strychnine. $P < 0.001$ by two-way ANOVA. *E*: AUC of the glucose-stimulated insulin perifusion in the absence or presence of 1 μmol/L strychnine. $^{**}P < 0.01$ compared with control 300 μmol/L glycine (*B*) or 0 glycine (*C*).

secretion (23 ± 8%-fold change compared with control, *n* = 18 replicates, 6 donors) (Fig. 5*C*) from intact islets. Because interstitial glycine concentrations are expected to exceed the circulating level, particularly because β-cells store and release glycine (see below), we also stimulated intact islets with 800 µmol/L glycine. This concentration, which is achieved in circulation upon oral glycine supplementation (18), further increased insulin secretion (34 ± 12%-fold change, *n* = 18 replicates, 6 donors; *P* < 0.01) (Fig. 5*C*) compared with control. Antagonism of the GlyR with 1 µmol/L strychnine prevented glycine-dependent insulin secretion in intact islets at 300 and 800 µmol/L of glycine (Fig. 5*C*). We further investigated the role for endogenous glycine signaling in insulin secretion by examining the effect of strychnine on dynamic insulin secretion responses to high glucose (Fig. 5*D*). Strychnine reduced glucose-stimulated insulin secretion from human islets (*n* = 10 replicates, 5 donors; *P* < 0.001 by two-way ANOVA; area under the curve *P* = 0.054) (Fig. 5*E*).

Glycine Release From Human β-Cells

Rat pancreatic islets contain ~6 mmol/L glycine (29). Immunohistochemistry and immunogold electron microscopy show that rat β-cells express the vesicular amino acid transporter (VIAAT/VGAT), which mediates glycine uptake into synaptic vesicles in neurons and accumulates glycine in secretory granules (30). These findings, and the ability of strychnine to inhibit insulin secretion from human islets (above), suggest that β-cells may release glycine by exocytosis to mediate autocrine signaling. By fluorescence immunohistochemistry we find that human islet cells express both of the GlyT, GlyT1 and GlyT2, which appear to localize both to β- and non–β-cells (Fig. 6*A* and *B* and Supplementary Fig. 5). We investigated the release of glycine from β-cells by adapting a patch-clamp–based assay that has previously been used to detect the exocytotic release of γ-aminobutyric acid (GABA) and ATP (3,31,32). Isolated human islet cells were transfected with a plasmid vector overexpressing GlyRα1, leading to an approximate sevenfold increase of the glycine-evoked currents. These GlyR will sense glycine release from the same cells, giving rise to currents that are easily detected. Transfected cells were stimulated by infusing 2 µmol/L free Ca^{2+}. A representative experiment is shown in Fig. 6*Ci*. Superimposed on the background current were spontaneous transient inward currents (TICs). These activated rapidly (6.4 ± 2.5 ms 10–90% rise time, *n* = 7, 2 donors) and decayed more gradually, with a half-width of 10.5 ± 3.5 ms, reminiscent of inhibitory postsynaptic currents in neurons. The TICs were completely suppressed by strychnine (Fig. 6*Cii*), suggesting that each TIC reflects the exocytosis of a glycine-containing vesicle.

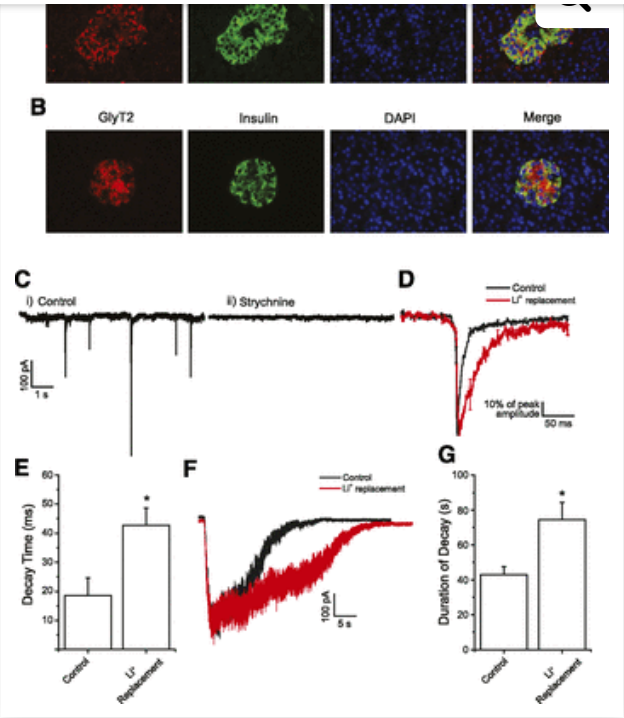

Figure 6

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Glycine secretion from human β-cells and GlyT activity. Human pancreatic tissue sections were immunostained for GlyT1 (*A*) (red), GlyT2 (*B*) (red), and insulin (green). Nuclei were stained with DAPI (blue). *C*: β-Cells overexpressing GlyRα1 subunits were clamped at −70 mV and infused with an intracellular solution containing ~2 µmol/L free Ca^{2+}. TIC firing (*i*) was suppressed in the presence of 1 µmol/L strychnine (*ii*). *D*: Summary trace of glycine TIC from control cells and after replacement of Na^+ with Li^+ to block GlyT activity. *E*: Total decay time from TIC. *F*: Sample trace of stopped-flow experiments to assess glycine clearance from control cells and after replacement of Na^+ with Li^+ to block GlyT activity. *G*: Total decay time from stopped-flow experiments. **P* < 0.05 compared with control.

The glycine concentration of the cerebrospinal fluid is ~5 µmol/L (33), whereas glycine levels in the plasma are ~40-fold higher (150–400 µmol/L) (8,18). These plasma glycine levels are above the half-maximal effective concentration (EC_{50}) of the GlyR in β-cells (see above), and ligand-gated ion channels desensitize during prolonged exposure to high agonist concentrations. Two techniques were used to determine whether β-cells clear glycine through the activity of GlyT to maintain a low intraislet glycine concentration. First, we investigated clearance after endogenous glycine release. Because GlyT require Na^+ to cotransport glycine, extracellular Na^+ was replaced with Li^+ to effectively inhibit both GlyT1 and GlyT2 (34). β-Cells overexpressing GlyRα1 were stimulated to secrete glycine in the presence and absence of Na^+, and glycine clearance was characterized. Figure 6*D* shows an averaged TIC event, with and without replacement of Na^+ with Li^+ (*n* = 7 cells, 21 events, 2 donors for control; *n* = 5 cells, 58 events, 2 donors for Li^+ replacement). Li^+ replacement significantly prolonged the total decay time of the glycine TIC from 18 ± 6 to 42 ± 6 ms (*P* < 0.05) (Fig. 6*E*).

Second, we investigated the clearance of exogenous glycine in a stopped-flow experiment (34). After fast local application of 100 µmol/L glycine, rather than applying an extracellular solution to

and GlyT activity. Figure 6*F* shows a sample trace of a stopped-flow experiment. Following Li^+ replacement, cells required more time (74 ± 9 s) to return to baseline compared with their controls (43 ± 4 s, n = 10 cells, 3 donors; $P < 0.05$) (Fig. 6*G*).

Discussion

In the current study, we found that GlyR and GlyT are highly expressed in human β-cells and that activation of GlyR stimulates electrical activity, increases $[Ca^{2+}]_i$, and enhances insulin secretion. GlyR in human islets is recently reported to be expressed specifically in α-cells but not β-cells (35), supported by the finding that glycine stimulated glucagon but not insulin secretion from human islets. However, the experiments were performed in the absence of glucose, which leads to the activation of unphysiologically large K_{ATP} currents in β-cells and may prevent any glycine effect on the membrane potential. Li et al. (35) observed $[Ca^{2+}]_i$ responses upon glycine application in a subset of dispersed islet cells, but an unequivocal identification of the cell types was not performed. Our data clearly demonstrate that among human islet cells, GlyR and glycine-activated Cl^- currents are most active in β-cells.

The effect of glycine on $[Ca^{2+}]_i$ in β-cells was blocked by the GlyR antagonist strychnine, demonstrating that it was not secondary to Na^+-dependent uptake of amino acid, as previously reported for mouse β-cells (36). GlyR current activity was significantly lower in mouse β-cells compared with human β-cells and was undetectable in rat β-cells. This observation adds to the previously reported differences between human and rodent islets (25,37,38). It can be speculated that this reflects differences in the diets: the glycine content of meat (6.5% of amino acids [39]) is more than double that of wheat protein (2.8% amino acids [40]).

Unlike GlyR in the central nervous system, which are inhibitory, we demonstrate that pancreatic GlyR activation increased $[Ca^{2+}]_i$ in most β-cells but had an inhibitory effect in a small population of cells. How can these divergent effects be explained? Activation of Cl^--permeable GlyR will drive the membrane potential toward the Cl^- equilibrium potential (E_{Cl}). We have previously reported that E_{Cl} in β-cells is ~−40 mV, which is above the threshold for electrical activity (1). Our data suggest that some β-cells were depolarized beyond E_{Cl} before glycine addition and that glycine consequently hyperpolarized rather than depolarized these cells and thereby decreased $[Ca^{2+}]_i$. In support of this, cells responding with a decrease in $[Ca^{2+}]_i$ displayed a significantly higher baseline $[Ca^{2+}]_i$ than cells that responded with an increase. The positive E_{Cl} compared with neurons (<–60 mV) reflects the higher intracellular Cl^- concentration in β-cells (41).

The plasma glycine concentration is ~40-fold higher than that of the cerebrospinal fluid and above the EC_{50} for GlyR activation. Indeed, we believe the local extracellular glycine concentration in islets is significantly lower due to the GlyT-dependent clearance of glycine. These transporters are very effective at clearing glycine (V_{max} = 379 pmol/min/mg for GlyT1 and

depolarizations of β-cells [34]). Our experiments show that inhibition of GlyT via Li^+ replacement results in nearly a doubling of the endogenous glycine signal, suggesting that GlyT not only clear plasma glycine but also regulate glycine signaling in cells. Likewise, exogenous application of glycine in stopped-flow experiments demonstrated a significant loss of glycine clearance during GlyT inhibition, and this is likely exaggerated within the confined interislet cell space as opposed to the isolated cells studied here.

We examined whether glycine is secreted from β-cells by performing patch-clamp experiments in cells overexpressing GlyR, which serve as sensors for glycine released from the same cell (i.e., an autosynapse). Although the experiments should in principle be feasible in untransfected cells, overexpression of GlyR improves the sensitivity of the assay considerably. We found that Ca^{2+} infusion elicited TICs that reflect the exocytotic release of a GlyR-activating compound. It is likely that this compound mainly represents glycine secreted from insulin granules, synaptic-like microvesicles, or both (30), but we cannot exclude taurine, which is likewise present in high concentrations in the islets (29). Similar to GABA and $GABA_A$ receptors (1) and ATP and P2 receptors (2,3), autocrine activation of GlyR may constitute a positive autocrine feedback loop in human β-cells.

Ingestion of glycine reduces blood glucose levels (18), and it was suggested that glycine was stimulating insulin secretion in humans. Although physiological glycine (300 μmol/L) only tended to increase insulin secretion, glycine at 800 μmol/L significantly increased insulin secretion. We believe that in the insulin secretion studies where islets were intact, the cells in the core of the islets do not experience the same glycine concentrations as the cells on the exterior, explaining the discrepancy in glycine concentrations to elicit a response. In addition to demonstrating the ability of glycine to stimulate insulin, we demonstrated that insulin also feeds back to amplify the glycine current and that this requires phosphoinositide 3-kinase activation, which is downstream from the insulin receptor. Although we did not attempt to use insulin receptor antagonists, our findings are consistent with the insulin receptor–dependent amplification of glycine current in mouse spinal neurons (27).

Finally, we demonstrated that β-cells from donors with T2D have a significantly lower GlyR expression, lower glycine-activated Cl^- current than β-cells from donors without T2D, and are refractory to the effects of insulin, perhaps implicating β-cell insulin resistance as contributing to the reduced GlyR expression in T2D. The exact mechanism for GlyRα1 downregulation in these cells is unclear but could be related to impaired trafficking or activity in the face of β-cell insulin resistance or downregulation of protein levels downstream of mRNA expression, which was increased in T2D (Supplementary Fig. 1*B*).

In summary, we demonstrate here that GlyR Cl^- channels are highly expressed in human β-cells and contribute to the regulation of electrical activity and $[Ca^{2+}]_i$ signaling through an autocrine

contributing to impaired insulin secretion in T2D.

Article Information

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Footnotes

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