

γ -Aminobutyric Acid (GABA) Is an Autocrine Excitatory Transmitter in Human Pancreatic β -Cells

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OBJECTIVE—Paracrine signaling via γ -aminobutyric acid (GABA) and GABA_A receptors (GABA_ARs) has been documented in rodent islets. Here we have studied the importance of GABAergic signaling in human pancreatic islets.

RESEARCH DESIGN AND METHODS—Expression of GABA_ARs in islet cells was investigated by quantitative PCR, immunohistochemistry, and patch-clamp experiments. Hormone release was measured from intact islets. GABA release was monitored by whole-cell patch-clamp measurements after adenoviral expression of $\alpha_1\beta_1$ GABA_AR subunits. The subcellular localization of GABA was explored by electron microscopy. The effects of GABA on electrical activity were determined by perforated patch whole-cell recordings.

RESULTS—PCR analysis detected relatively high levels of the mRNAs encoding GABA_AR α_2 , β_3 , γ_2 , and π subunits in human islets. Patch-clamp experiments revealed expression of GABA_AR Cl⁻ channels in 52% of β -cells (current density 9 pA/pF), 91% of δ -cells (current density 148 pA/pF), and 6% of α -cells (current density 2 pA/pF). Expression of GABA_AR subunits in islet cells was confirmed by immunohistochemistry. β -Cells secreted GABA both by glucose-dependent exocytosis of insulin-containing granules and by a glucose-independent mechanism. The GABA_AR antagonist SR95531 inhibited insulin secretion elicited by 6 mmol/l glucose. Application of GABA depolarized β -cells and stimulated action potential firing in β -cells exposed to glucose.

CONCLUSIONS—Signaling via GABA and GABA_AR constitutes an autocrine positive feedback loop in human β -cells. The presence of GABA_AR in non- β -cells suggests that GABA may also be involved in the regulation of somatostatin and glucagon secretion. *Diabetes* 59:1694–1701, 2010

Pancreatic islets of Langerhans are endocrine micro-organs with a central role in plasma glucose homeostasis. Islets consist of three main endocrine cell types: insulin-producing β -cells, glucagon-secreting α -cells, and somatostatin-releasing δ -cells. Insulin release from β -cells is directly controlled by the blood glucose level and modulated by circulating hor-

mones and the autonomous nervous system. In addition, hormone release from β -cells, as well as from the other islet cell types, is regulated by autocrine and paracrine interactions.

The local signaling roles of the major islet hormones have been extensively studied and are well established (1). Islet cells also contain and release a variety of additional factors with putative local signaling functions, including ions (Zn²⁺, Ca²⁺) and neurotransmitters (GABA, glutamate, ATP) (2–5). We and others have presented evidence that GABA released from β -cells inhibits glucagon secretion in rodent islets by activating GABA_A receptors (GABA_AR) in α -cells (6–8).

The architecture of human islets, with non- β -cells distributed throughout the islet, rather than confined to the islet periphery as in rodents, facilitates paracrine signaling (9,10). Human β -cells contain high concentrations of GABA (11,12), and expression of GABA_AR subunits in human islets has been detected by RT-PCR (8,13). We have now studied the possible involvement of GABA/GABA_AR-mediated signaling in the regulation of hormone release from human islets. Our results suggest that GABA plays a more diverse role in human islets than suggested by previous work in rodent islets (5–7).

RESEARCH DESIGN AND METHODS

Islet preparation and cell culture. Human pancreases were obtained with ethical approval and clinical consent from nondiabetic donors. Islets were isolated in the Diabetes Research and Wellness Foundation Human Islet Isolation Facility by collagenase digestion (Serva, Heidelberg, Germany), essentially as reported previously (14,15). For hormone release measurements, the islets were cultured overnight in Connaught Medical Research Laboratories (CMRL) medium containing 5 mmol/l glucose. The electrophysiological experiments were performed on single cells or cell clusters obtained by dissociation of islets in Ca²⁺-free buffer (16). The resulting cell suspension was then plated onto plastic Petri dishes and cultured in RPMI-1640 containing 10 mmol/l glucose and 2 mmol/l L-glutamine. For biophysical detection of GABA release (Figs. 3–5), cells were infected with recombinant adenoviruses encoding the GABA_AR α_1 and β_1 subunits 24–48 h before the experiments (17).

Electrophysiology. Patch pipettes were pulled from borosilicate glass and heat-polished (tip resistance 4–8 M Ω). Petri dishes were mounted onto an Axiovert 10 microscope (Zeiss, Jena, Germany) positioned on a vibration isolation table with a Faraday cage (TMC, Peabody, MA). Experiments were performed in the standard or perforated-patch whole-cell configuration using an EPC9 amplifier and Pulse software (HEKA, Lambrecht, Germany). All electrophysiological measurements were conducted at 32–33°C and cells were continuously superfused with extracellular medium. For rapid application of GABA (Figs. 1 and 7), a Nanoliter 2000 Oocyte Injector (WPI, Stevenage, U.K.) was used.

The extracellular solution for measuring glucose- or tolbutamide-induced GABA release (Fig. 4) and membrane potential (Fig. 7) contained (in mmol/l) 138 NaCl, 5.6 KCl, 2.6 CaCl₂, 1.2 MgCl₂, 5 HEPES (pH 7.4, NaOH), and glucose at the indicated concentration. For detecting expression of endogenous GABA-activated currents (Fig. 1) and GABA-release elicited by Ca²⁺ infusion or voltage-clamp depolarizations in infected cells (Figs. 3 and 5), TEACl (20 mmol/l) was added and NaCl correspondingly reduced. In Fig. 1, the intracellular solution was composed of (in mmol/l) 120 CsCl, 1 MgCl₂, 10 EGTA, 1 CaCl₂, 10 HEPES, and 3 MgATP (pH 7.2, CsOH). In Fig. 4, CsCl was replaced

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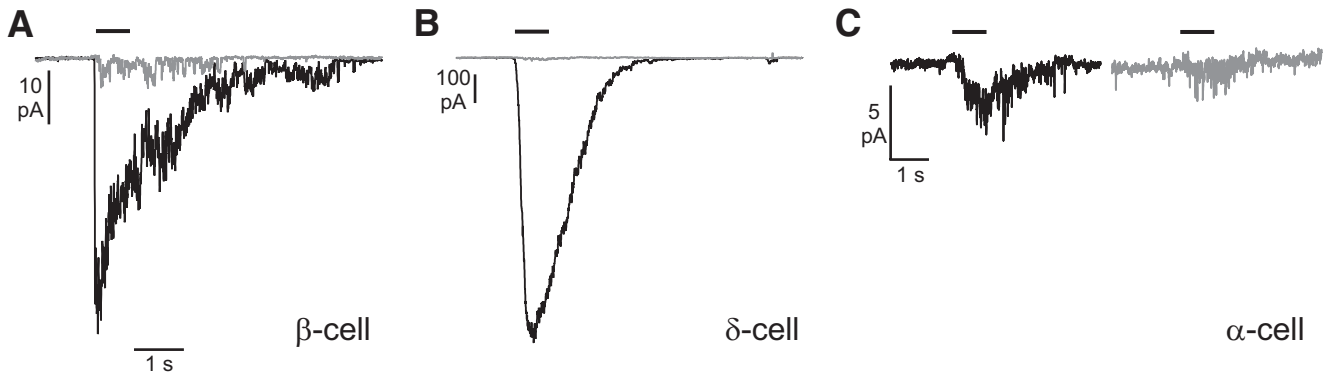


FIG. 1. Functional detection of endogenous GABA_AR Cl⁻ channels in human islet cells. **A:** Patch-clamp recording of currents evoked by puff application of GABA (1 mmol/l, as indicated by the bars) to an identified β -cell in the absence (*black trace*) and presence (*gray trace*) of 50 μ mol/l SR-95531 in the same cell. The cell was held at -70 mV throughout the experiment. **B:** As in **A**, showing a δ -cell (note the difference in scale bars). **C:** As in **A**, showing an α -cell. The cell had been incubated in the presence of 0.5 μ mol/l insulin for 1 h before the experiment.

equimolarly with KCl. The Ca²⁺ infusion experiments (Figs. 3A and 5A) were performed with pipette solution consisting of (in mmol/l) 110 CsCl, 10 KCl, 10 NaCl, 1 MgCl₂, 3 MgATP, 0.1 cAMP, 5 HEPES, 9 CaCl₂, and 10 EGTA (pH 7.15 with CsOH; free Ca²⁺ ~ 2 μ mol/l). Depolarization-evoked GABA release was recorded with intracellular medium containing (in mmol/l) 125 Cs-glutamate, 10 CsCl, 10 NaCl, 1 MgCl₂, 5 HEPES, 50 μ mol/l EGTA, 3 MgATP, and 0.1 cAMP (pH 7.15 with CsOH). The membrane potential measurements were performed with a pipette solution composed of (in mmol/l) 76 K₂SO₄, 10 KCl, 10 NaCl, 1 MgCl₂, 5 HEPES, and 10–50 μ g/ml gramicidin (pH adjusted to 7.35 with CsOH, osmolarity adjusted to ~ 300 mOsm with sucrose). Biocytin (0.5 mg/ml) was sometimes added to the pipette solution to facilitate subsequent immunocytochemical identification.

Amperometry. Cells were preloaded with serotonin by addition of 0.5 mmol/l 5-hydroxytryptophan and 0.5 mmol/l serotonin to the culture medium >6 h before the experiment. Serotonin release was detected using a carbon fiber electrode (ProCFE, Dagan Corporation, Minneapolis, MN) connected to the second head-stage of the EPC9/3 amplifier. The electrode was held at 650 mV and positioned ~ 1 μ m from the cell. Exocytosis was stimulated by infusion of cells with solution containing 2 μ mol/l free Ca²⁺ via the patch electrode (see above), and whole-cell GABA-activated currents were measured in parallel from the same cell.

Immunocytochemistry. De-paraffinized human pancreatic tissue sections (4 μ m) were heated to 95°C in 10 mmol/l Tris + 1 mmol/l EDTA (pH 6) for 40 min, followed by incubation at 4°C for 10 min in the same buffer. Sections

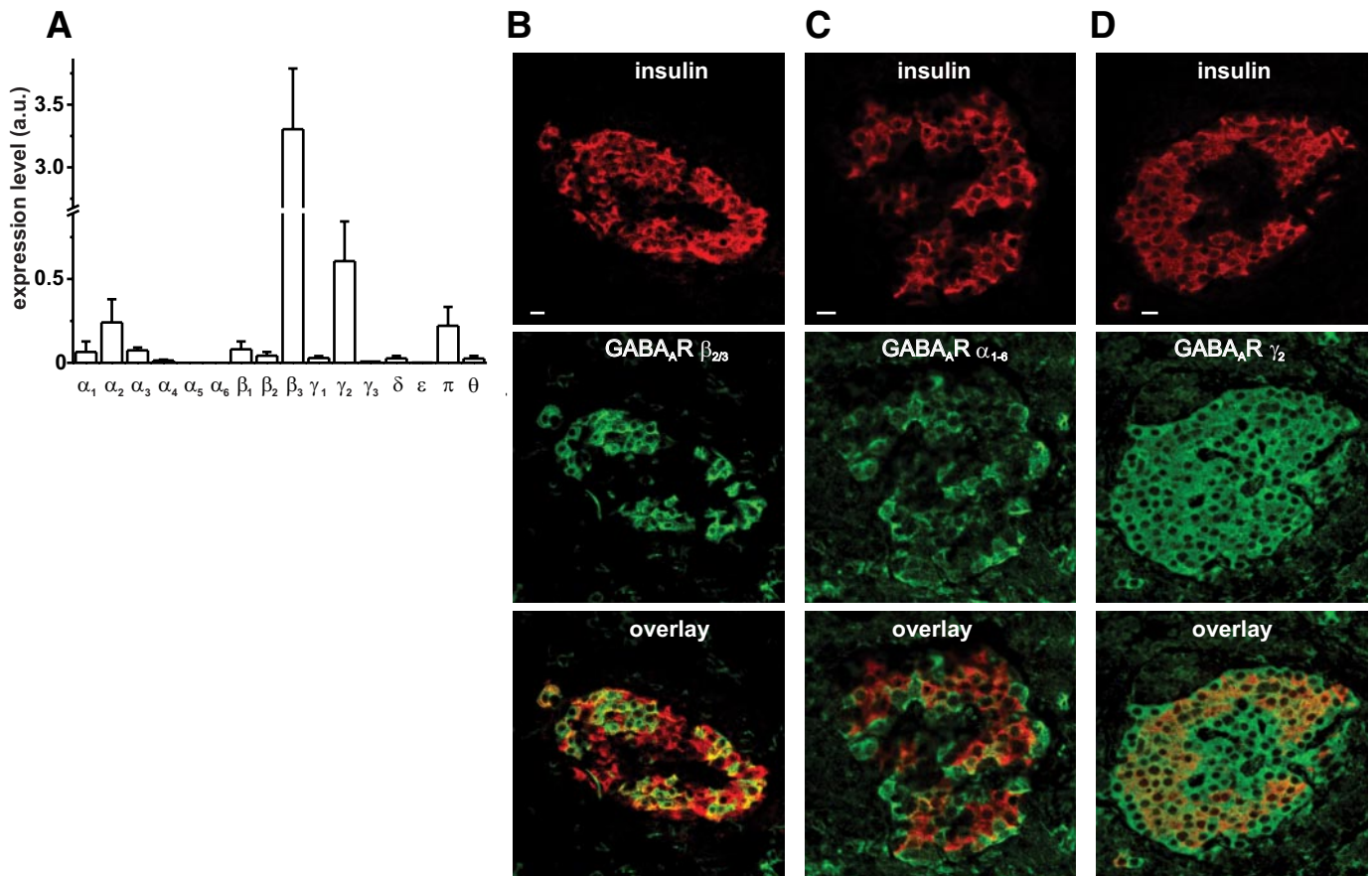


FIG. 2. Expression of GABA_AR subunits in human islets. **A:** Expression profiling of GABA_AR subunits in islets by quantitative RT-PCR ($n = 3$ preparations from three donors). **B–D:** Co-labeling of pancreatic tissue sections with anti-insulin and anti-GABA_AR $\beta_{2/3}$ (**B**), anti-GABA_AR α_{1-6} (**C**), or anti-GABA_AR γ_2 (**D**). Insulin is shown in red and GABA_AR subunits in green; co-localization results in yellow labeling (scale bars = 10 μ m). a.u., arbitrary units.

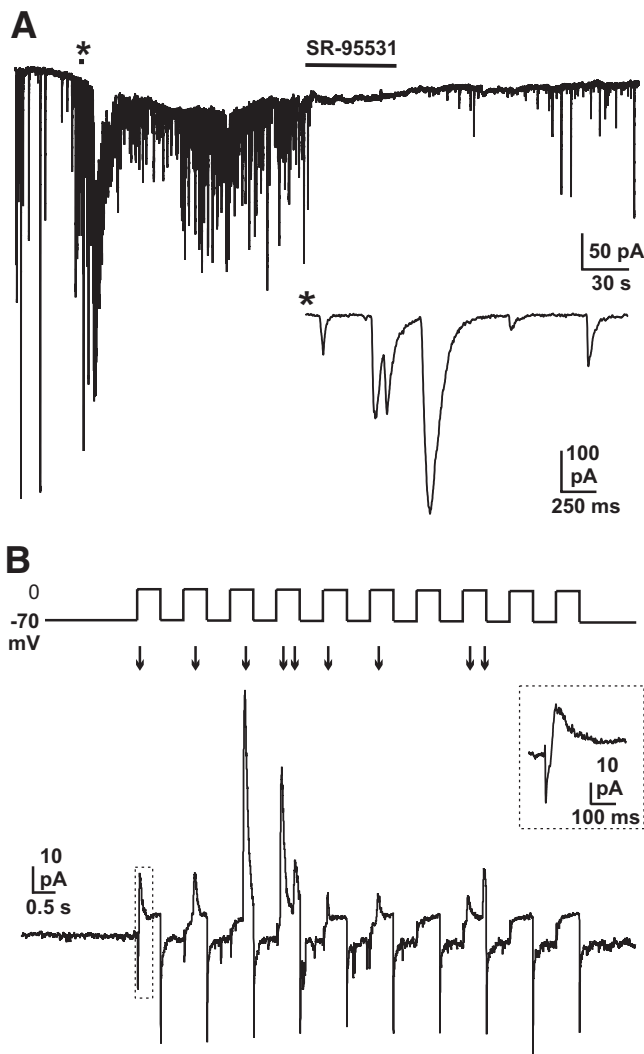


FIG. 3. Quantal release of GABA from human β -cells. **A:** A β -cell overexpressing α_1/β_1 GABA_AR was held at -70 mV and infused with intracellular solution containing $2 \mu\text{mol/l}$ free Ca^{2+} (at 5 mmol/l extracellular glucose). SR-95531 ($10 \mu\text{mol/l}$) was applied as indicated by the bar. The inset shows a part of the trace (indicated by *) on an expanded time scale. **B:** A train of 10 500-ms voltage-clamp depolarizations from -70 to 0 mV was applied to a cell overexpressing α_1/β_1 GABA_AR (with intracellular solution containing $50 \mu\text{mol/l}$ EGTA). GABA-induced transient currents are indicated by arrows. The inset shows a part of the trace (marked by the dotted rectangle) on an expanded time base. Note that the direction of transient currents is outward at 0 mV. The initial downward component represents the opening of the voltage-gated Na^+ and Ca^{2+} currents triggered by the depolarization. The activation of the GABA_AR accounts for the outward current. The recording shown is representative of seven experiments.

were then incubated with antibodies directed against GABA_AR subunits α_{1-6} (1:50, H-300; Santa Cruz Biotechnology, Santa Cruz, CA), $\beta_{2/3}$ (1:100, MAB341, Millipore, Hampshire, U.K.) or γ_2 (1:100, LS-C14, Life span, Seattle, WA) dissolved in REAL antibody diluent (Dako, Cambridge, U.K.) at 4°C overnight. Endogenous peroxidases were blocked using methanol containing 1% H_2O_2 (15 min). A peroxidase-conjugated secondary antibody (Dako) was added for 30 min, followed by Alexa 488-labeled tyramide (Invitrogen) for 20 min. Co-staining for insulin, glucagon, somatostatin, or pancreatic polypeptide and visualization of fluorescence were performed as previously described (18).

Identification of the cell type after patch-clamp experiments was performed by immunodetection of insulin, glucagon, and somatostatin as described previously (19,20) (see the online appendix available at <http://diabetes.diabetesjournals.org/cgi/content/full/db09-0797/DC1>).

Hormone secretion measurements. Batches of 10 – 20 islets (in triplicates) were preincubated in 1 ml Krebs-Ringer buffer containing 2 mg/ml BSA and supplemented with 1 mmol/l glucose for 1 h followed by a 1 -h test incubation

in 1 ml Krebs-Ringer buffer supplemented as indicated. The hormone content of the supernatant was measured by radioimmunoassay (insulin, glucagon: Millipore, Watford, U.K.; somatostatin: Euro-Diagnostica, Malmö, Sweden).

Quantitative RT-PCR. Gene expression profiling of GABA_AR subunits was performed by RT-qPCR on human islet total RNA as described previously (19) (see the online appendix).

Electron microscopy. Isolated human islets were fixed in 2.5% glutaraldehyde in phosphate buffer, postfixed in 1% OsO_4 , dehydrated, and embedded in Spurr's resin. Ultrathin sections cut onto nickel grids were immunolabeled for GABA using a rabbit polyclonal antibody ($1:1,000$ dilution, Sigma) and protein A gold particles (15 nm, Biocell, Cardiff, U.K.). Sections were viewed with a Joel 1010 microscope (accelerating voltage 80 kV).

Data analysis. All data are expressed as means \pm SEM. Statistical significances were calculated using Student's *t* test. GABA-induced transient inward currents (TICs) and amperometric events were analyzed using MiniAnalysis software (Synaptosoft, Decatur, GA).

RESULTS

Expression of endogenous GABA_AR Cl^- channels in human islets. Functional expression of endogenous GABA_AR Cl^- channels in human islet cells was investigated in patch-clamp experiments. Figure 1A shows a representative recording in which puff application of GABA to a β -cell triggered a rapidly activating and desensitizing inward current that was sensitive to the GABA_AR antagonist SR-95531. GABA-activated currents were found in 26 of 50 β -cells (52%), and the current amplitude in receptor-positive cells averaged 64 ± 20 pA (current density 9.4 ± 2.1 pA/pF). The GABA-evoked current was decreased by $78 \pm 2\%$ in the presence of SR-95531 (from 32 ± 5 to 7 ± 2 pA, $P < 0.01$, $n = 8$). GABA application also elicited inward currents in 31 out of 34 δ -cells (91%), but the responses were typically much larger than in β -cells (Fig. 1B). On average, the GABA-evoked currents had an amplitude of 668 ± 188 pA (148 ± 42 pA/pF, $n = 31$), which was reduced by $75 \pm 6\%$ (from 570 ± 330 to 33 ± 8 pA, $P < 0.01$, $n = 10$) in the presence of SR-95531. By contrast, GABA application triggered inward currents in only 3 out of 48 α -cells (6% ; Fig. 1C), with the current amplitude in these cells averaging 7 ± 3 pA (current density 2 pA/pF). Insulin has been reported to translocate GABA_AR to the cell surface in α -cells (8). In α -cells that had been preincubated with insulin ($0.5 \mu\text{mol/l}$ for 1 h in the culture medium), application of GABA in the continued presence of insulin ($0.1 \mu\text{mol/l}$) elicited inward currents in 3 out of 19 cells tested (16%), but the current amplitude was not increased and averaged 4.3 ± 0.6 pA/pF in these three cells. As shown in Fig. 1C, SR95531 reduced the GABA-activated current in α -cells by 70% ($n = 2$). Of the 64 cells analyzed in which GABA evoked a measurable current, only one cell did not contain insulin, glucagon, or somatostatin, and this cell was not positive for pancreatic polypeptide (not shown).

Expression of GABA_AR subunits in human islets was investigated by quantitative RT-PCR. Among the α - and β -subunits, α_2 and β_3 predominated. Fairly high levels of the γ_2 and π were also observed. In addition, low levels of α_1 , α_3 , α_4 , β_1 , β_2 , γ_1 , and θ were detected (Fig. 2A).

Expression of GABA_AR subunits in human islets was confirmed by immunohistochemistry. An antibody against the $\beta_{2/3}$ subunit labeled $\sim 50\%$ of the β -cells in pancreatic tissue sections (Fig. 2B); no staining of α -cells, δ -cells, or PP-cells was observed (supplementary Fig. 1, available in the online appendix). An α_{1-6} -specific antibody stained 22% of the β -cells (Fig. 2C), 94% of the δ -cells, and 14% of the α -cells (supplementary Fig. 1). Finally, an antibody directed against the γ_2 subunit labeled β -cells (Fig. 2D) as well as α - and δ -cells (supplementary Fig. 1). No specific

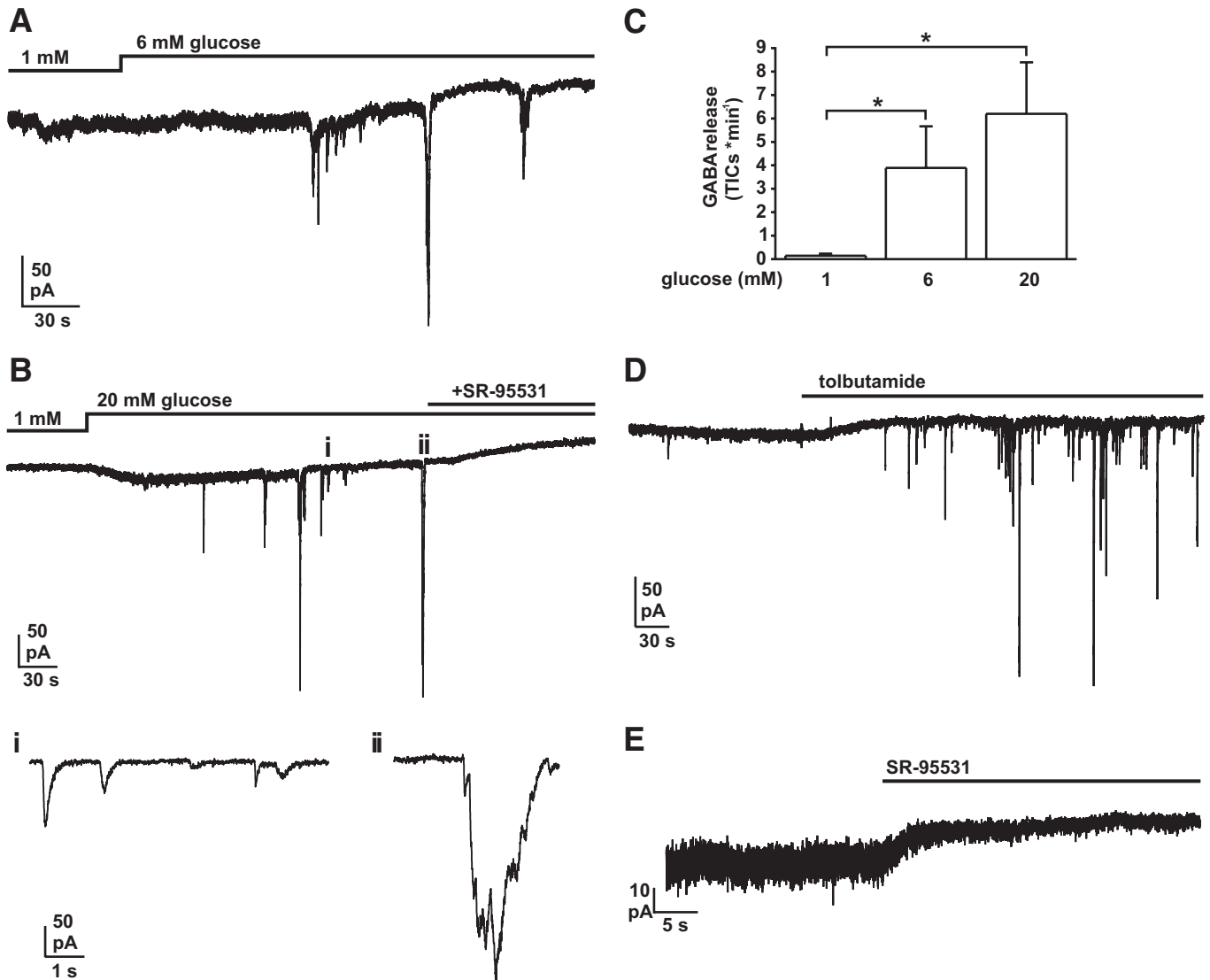


FIG. 4. Glucose- and tolbutamide-induced GABA release from human β -cells. Experiments were performed in small clusters of islet cells overexpressing α_1/β_1 GABA_AR. The patch-clamped cell was held at -70 mV and infused with pipette solution containing 10 mmol/l EGTA. **A:** The glucose concentration in the bath was increased from 1 to 6 mmol/l as indicated. **B (upper):** The extracellular glucose concentration was increased from 1 to 20 mmol/l. SR-95531 (10 μ mol/l) was included as indicated. **B (lower):** Sections of top trace (as indicated by letters *i* and *ii*) shown on an expanded time base. **C:** Summary of observed frequencies of GABA release (TICs/min) at the indicated glucose concentrations ($*P < 0.05$). The measurements were made at steady state (1–5 min after addition of glucose). **D:** Tolbutamide (100 μ mol/l) was applied as indicated (extracellular glucose concentration 4 mmol/l). **E:** SR-95531 (10 μ mol/l) was applied as indicated at 1 mmol/l extracellular glucose.

labeling of human pancreatic sections was observed with an antibody against β_1 subunits (not shown).

GABA release from human β -cells. To determine if GABA is released from human β -cells, a patch-clamp-based assay was used (17). Briefly, GABA_AR were overexpressed in isolated β -cells using adenoviral vectors encoding the GABA_AR α_1 and β_1 subunits, respectively. GABA released upon fusion of GABA-containing secretory vesicles activates these receptors in the same cell, giving rise to transient inward currents (TICs). Figure 3A shows a recording from a cell held at -70 mV, in which exocytosis was stimulated by inclusion of 2 μ mol/l free Ca^{2+} in the intracellular solution. The trace displays numerous TICs that were sensitive to the GABA_AR blockers SR-95531 (10 μ mol/l; $n = 4$) and bicuculline (100 μ mol/l; $n = 2$; not shown), demonstrating that they reflect activation of GABA_AR. The TICs had an average rise time (10–90%) of 14 ± 1 ms and a half-width of 40 ± 2 ms ($n = 274$ events

from four cells). These values are comparable to those reported in rat β -cells (17).

Quantal release of GABA could be elicited by voltage-clamp depolarizations from -70 to 0 mV (Fig. 3B). Depolarization-evoked transient currents were outward because the experiments were conducted at low $[\text{Cl}^-]_i$ (22 mmol/l). Thus, the Cl^- equilibrium potential (E_{Cl}) is approximately -50 mV, resulting in a net inward driving force at 0 mV for Cl^- ions.

GABA release was also observed in δ -cells stimulated by Ca^{2+} infusion or voltage-clamp depolarizations (supplementary Fig. 2A). As δ -cells express high levels of endogenous GABA_AR, GABA-induced TICs were sometimes (three cells) seen even in noninfected cells (supplementary Fig. 2B).

Glucose-induced GABA release. Figure 3B demonstrated that GABA is released from β -cells in response to depolarization-induced Ca^{2+} influx via voltage-gated Ca^{2+}

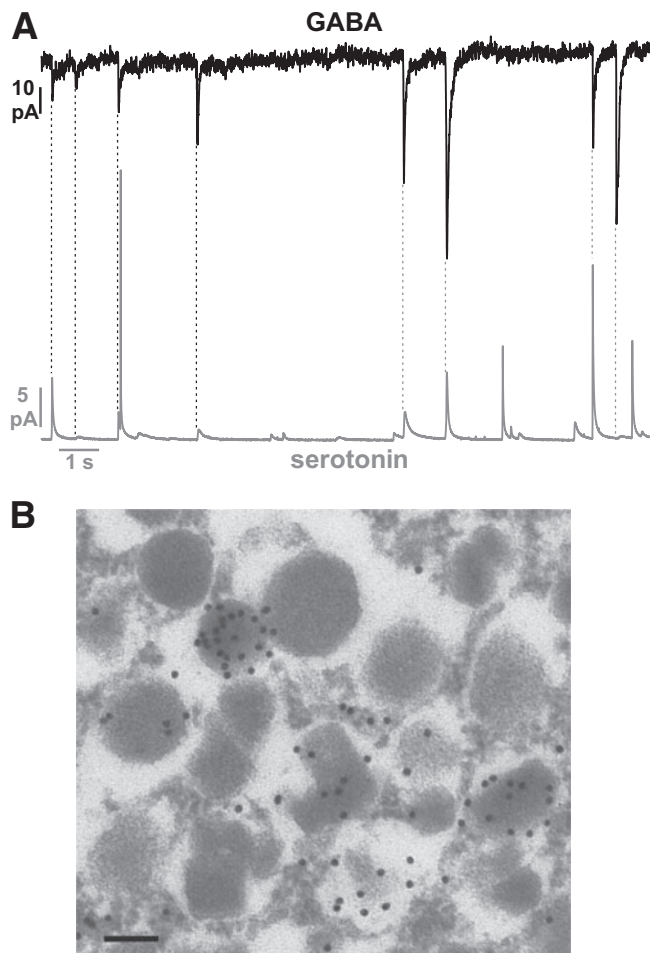


FIG. 5. Storage and secretion of GABA by insulin-containing LDCVs in human β -cells. **A:** GABA release was detected by patch-clamping in an identified β -cell overexpressing α_1/β_1 GABA_AR (upper trace). The cell was held at -70 mV and infused with intracellular solution containing $2 \mu\text{mol/l}$ free Ca^{2+} . Serotonin release was measured simultaneously in the same cell by carbon fiber amperometry (lower trace). The dashed lines indicate simultaneous occurrence of GABA-induced TICs and amperometric currents. **B:** Immunogold labeling of GABA in a human β -cell. Scale bar: 250 nm.

channels. We went on to investigate whether glucose, via membrane depolarization, can evoke GABA release. These experiments were performed on clusters of islet cells overexpressing α_1/β_1 GABA_AR. The membrane potential of the patch-clamped cell was held at -70 mV and its cytoplasm infused with a buffer containing 10 mmol/l EGTA. These steps prevent exocytosis in the patch-clamped cell, and it will therefore serve as a sensor ("sniffer-cell") for GABA released from neighboring cells within the cluster. TICs evoked by GABA release were rarely observed at 1 mmol/l extracellular glucose, but increasing the glucose concentration to 6 or 20 mmol/l increased the frequency of TICs after a delay of 1–2 min (Fig. 4A–C). The glucose-induced TICs were sensitive to SR-95531 (Fig. 4B), confirming that they reflect GABA release and activation of GABA_AR. When they occurred, the TICs tended to occur in bursts (Fig. 4B, *ii*).

GABA release was likewise stimulated by addition of the ATP-sensitive K^+ (K_{ATP}) channel blocker tolbutamide (100 $\mu\text{mol/l}$) to the extracellular solution (Fig. 4D; $n = 4$) and inhibited by the K_{ATP} channel opener diazoxide ($n = 4$, supplementary Fig. 3). The change in holding current in Fig. 4D probably reflects closure of K_{ATP} channels that

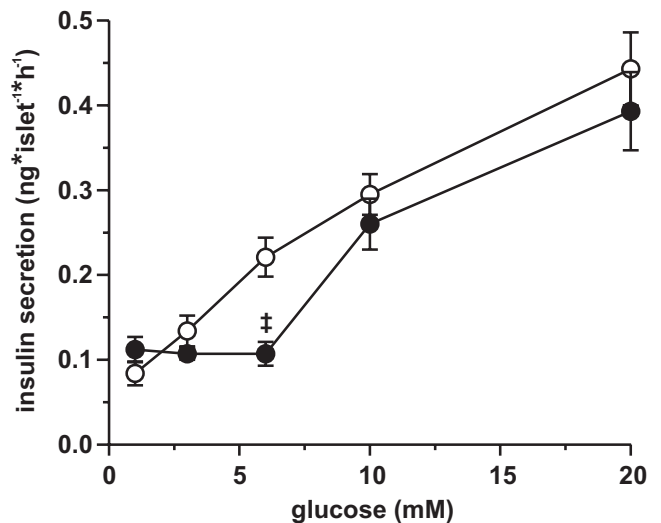


FIG. 6. Effect of GABA_AR blockade on insulin secretion from isolated human islets. Insulin secretion was measured at 1, 3, 6, 10, or 20 mmol/l extracellular glucose in the absence (○) or presence (●) of SR-95531 (10 $\mu\text{mol/l}$) as indicated ($n = 9$ –12 from four donors; $\ddagger P < 0.001$ for the effect of SR-95531). Under control conditions insulin release at all glucose concentrations was significantly different from the previous lower glucose concentration (not indicated). The results at 1, 6, and 20 mmol/l glucose were repeated with islets from seven different donors with the same results.

remain active in the patch-clamped cells despite the presence of 3 mmol/l ATP in the intracellular solution. By contrast, glucose stimulation did not consistently affect the holding current.

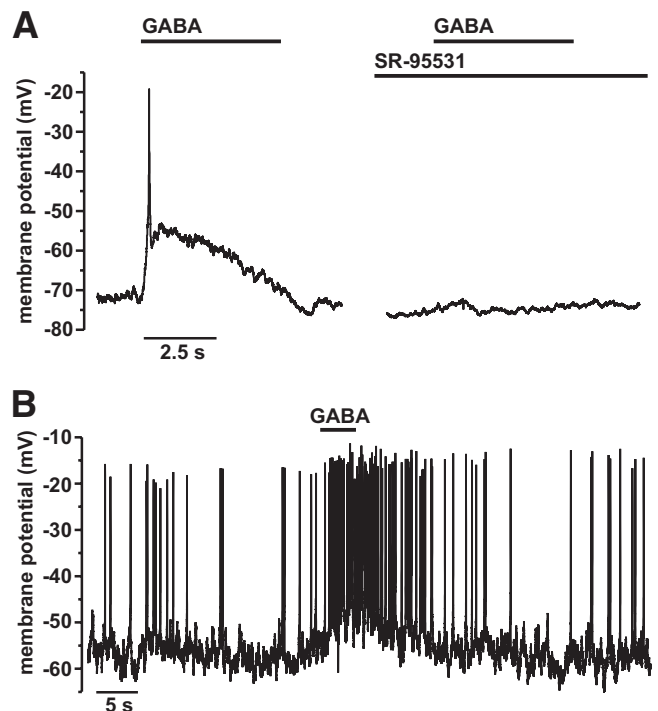


FIG. 7. Effects of GABA on the membrane potential of human β -cells. Membrane potential was recorded in noninfected cells in the perforated-patch configuration, using the Cl^- -impermeable antibiotic gramicidin as the perforating agent. Recordings were made at 6 mmol/l extracellular glucose. **A:** Application of GABA (100 $\mu\text{mol/l}$, indicated by bars) to a β -cell in the absence (left) or presence (right) of 50 $\mu\text{mol/l}$ SR-95531. **B:** Application of 10 $\mu\text{mol/l}$ GABA (bar) to an electrically active β -cell.

In cell clusters overexpressing α_1/β_1 GABA_AR, addition of SR-95531 reduced both the holding current and current noise even at low (1 mmol/l) extracellular glucose, in the absence of observable TICs (Fig. 4E). In a series of seven experiments, SR-95531 decreased the holding current from -47 ± 15 to -36 ± 15 pA ($P < 0.05$) and the current variance from $9.8 \pm 2.7 \times 10^{-24}$ A² to $4.1 \pm 1.8 \times 10^{-24}$ A² ($P < 0.05$). From the excess noise (sensitive to SR-95531), we estimate the size of the unitary event as ~ 0.5 pA, corresponding to a single-channel conductance of 7 pS. This is in reasonable agreement with the 10–15 pS reported for recombinant $\alpha_1\beta_1$ GABA_AR Cl⁻ channels (21).

Subcellular localization of GABA in human β -cells. GABA and GAD65 have been reported to be associated with synaptic-like microvesicles in rodent β -cells (22). However, we have recently presented evidence for release of GABA from insulin-containing large dense-core vesicles (LDCV) in rat β -cells (23). To determine whether vesicular GABA secretion from human β -cells reflects exocytosis of insulin-containing granules, we combined the patch-clamp-based assay for GABA release with amperometric detection of serotonin, assumed to accumulate in LDCVs (24–27). As shown in Fig. 5A, the transient currents induced by vesicular release of GABA (*top*) and serotonin (*bottom*) occurred simultaneously in most cases. In this experiment, 90 out of 165 GABAergic TICs were accompanied by a simultaneous amperometric spike. In a series of four experiments, co-release of serotonin was detected for $44 \pm 9\%$ of the GABA-release events. This suggests that GABA and serotonin are released by exocytosis of the same vesicles; the correlation is $< 100\%$ because amperometry only detects exocytotic events occurring in the vicinity of the carbon fiber (usually $< 50\%$), whereas GABA release is detectable over the entire cell surface (23). Close inspection of the records indicated that GABA may also be released during the “pedestals” that precede full fusion and during kiss-and-run exocytosis (supplementary Fig. 4).

Not all amperometric events were accompanied by simultaneous release of GABA (Fig. 5A). Indeed, only $13 \pm 3\%$ ($n = 4$) of the amperometric events were associated with GABA-evoked TICs, suggesting that GABA is only present in a subpopulation of insulin granules. This was verified by immunogold electron microscopy using a GABA-specific antibody. Some insulin granules were strongly labeled with gold particles, whereas other granules were not stained at all (Fig. 5B). This finding is in agreement with previous results from rat β -cells (23). Immunogold electron micrographs confirmed that non- β -cells (including δ -cells) contain GABA at levels 50–70% of those detected in β -cells (supplementary Fig. 5).

Effect of GABA_AR blockade on insulin secretion. In intact human islets, glucose at a concentration as low as 3 mmol/l stimulated insulin secretion above that seen at 1 mmol/l glucose. At higher glucose concentrations, there was a concentration-dependent further stimulation of insulin release (Fig. 6). The GABA_AR antagonist SR-95531 had no effect on basal insulin release but inhibited insulin release at 6 mmol/l glucose. No (statistically) significant inhibition of insulin secretion was observed at 3, 10, or 20 mmol/l glucose.

Somatostatin secretion was stimulated by elevation of the glucose concentration to ≥ 3 mmol/l (supplementary Fig. 6A). Glucagon secretion was suppressed when the glucose level in the medium was raised from 1 to ≥ 3 mmol/l (supplementary Fig. 6B). Inhibition was maximal

at 6 mmol/l glucose and gradually reduced at higher concentrations (28). SR95531 had complex and glucose-dependent effects on glucagon and somatostatin secretion.

Effect of endogenous GABA_AR activation on islet cell membrane potential. The effect of activation of endogenous GABA_AR on the membrane potential of islet cells was studied in noninfected cells using the perforated-patch whole-cell configuration. To maintain normal $[Cl^-]_i$, the Cl⁻-impermeable antibiotic gramicidin was used as the perforating agent (29). Figure 7A shows a β -cell exposed to 6 mmol/l glucose that was not electrically active. We point out that insulin secretion at 6 mmol/l is only $\sim 40\%$ of that evoked by 20 mmol/l glucose. The finding that some β -cells are not electrically active at the lower glucose concentration indicates that the concentration-dependent stimulation of insulin secretion involves recruitment of β -cells that were previously not active. Application of GABA depolarized the cell to approximately -55 mV. This effect was blocked by the GABA_AR antagonist SR-95531. On average, puff application of GABA depolarized β -cells from -64 ± 7 to -43 ± 4 mV ($n = 4$). Assuming that the membrane potential in the presence of GABA approximates E_{Cl} , $[Cl^-]_i$ can be estimated to be 32 ± 5 mmol/l ($E_{Cl} = -60 \cdot \log([Cl^-]_o/[Cl^-]_i)$). GABA, when applied at the high concentration used in Fig. 7A (100 μ mol/l), typically triggered single action potentials. This we attribute to the large Cl⁻ conductance, which effectively clamps the membrane potential to E_{Cl} and thus prevents regenerative electrical activity. When a lower GABA concentration (10 μ mol/l) was applied to a β -cell that was electrically active at 6 mmol/l glucose (Fig. 7B), a smaller membrane depolarization accompanied by increased action potential firing was observed. In a series of five experiments, 10 μ mol/l GABA increased β -cell action potential frequency approximately fivefold, from 0.6 ± 0.2 to 2.9 ± 0.4 Hz ($P < 0.05$).

Puffer application of GABA also depolarized the membrane potential of δ -cells (supplementary Fig. 7). In a series of four experiments, the δ -cell membrane potential averaged -28 ± 5 mV after application of 100 μ mol/l GABA, corresponding to a $[Cl^-]_i$ of 54 ± 9 mmol/l. The membrane potential of α -cells was not affected by GABA, reflecting the low GABA_AR density in these cells (not shown).

DISCUSSION

Human pancreatic islets contain concentrations of GABA similar to those found in the brain (30), but the physiological function of pancreatic islet GABA in humans remains poorly defined. Here we have investigated the role of GABA and GABA_AR Cl⁻ channels in paracrine and autocrine signaling in human pancreatic islets.

Blocking GABA_AR inhibited insulin secretion from human islets induced by 6 mmol/l glucose, i.e., close to the concentration at half-maximal stimulation (EC_{50}) for glucose-stimulated insulin secretion (31). These findings imply that GABA, by activating GABA_AR, stimulates β -cell secretion. This may seem paradoxical, since GABA is best known as an “inhibitory neurotransmitter.” GABA_ARs are ligand-gated Cl⁻ channels, and opening of these channels shifts the cell’s membrane potential toward E_{Cl} . The effect of GABA will accordingly depend on the membrane potential of the cell and $[Cl^-]_i$. Our measurements of E_{Cl} indicate that $[Cl^-]_i$ in human β -cells is ~ 32 mmol/l. This value is in close agreement with that measured in mouse

β -cells using a Cl^- -sensitive fluorescent indicator (34 mmol/l [32]). Assuming a plasma Cl^- concentration of 110 mmol/l, E_{Cl} in β -cells is greater than -40 mV and thereby positive to the threshold for action potential firing in human β -cells (-55 mV [19]). For comparison, $[\text{Cl}^-]_i$ in neurons is <10 mmol/l, resulting in an E_{Cl} more negative than -60 mV (33), whereas it will produce depolarization in β -cells exposed to nonstimulatory glucose concentrations. A stimulatory role for Cl^- conductances in β -cells is also supported by the observation that a reduction of $[\text{Cl}^-]_i$ below physiological levels suppresses glucose-induced electrical activity in mouse β -cells (34). A positive GABAergic feedback loop may account for bursts of TICs observed during glucose stimulation (Fig. 4B, *ii*): the release of one GABA-containing vesicle will depolarize the β -cell and this in turn (via stimulation of electrical activity) triggers the release of further vesicles. Desensitization of GABA_ARs eventually terminates the burst.

GABA_AR inhibition reduced insulin secretion at 6 but not 10 and 20 mmol/l glucose. This is similar to results obtained in INS-1 cells where GABA stimulates insulin secretion at 2.8 mmol/l glucose but is inhibitory at 28 mmol/l glucose (35). These divergent effects of GABA can be explained if increasing glucose concentrations lead to a progressive depolarization of the β -cell membrane potential (36,37). At high glucose levels, when the membrane potential approaches E_{Cl} , opening of Cl^- channels may have no further depolarizing effect and even reduce excitability (by clamping the membrane potential to E_{Cl}).

It has previously been demonstrated that glucose regulates GABAergic signaling in islets by increasing GABA_AR subunit expression (6) and via insulin-induced trafficking of GABA_ARs to the cell surface (8). Here we provide the important additional observation that glucose stimulates vesicular release of GABA from β -cells (Fig. 4C). GABA was co-released with the LDCV marker serotonin, and electron microscopy demonstrated accumulation of GABA in a subpopulation of insulin granules. This is in agreement with similar observations in rat β -cells (23). The findings suggest that vesicular GABA release in human β -cells is principally due to exocytosis of insulin-containing LDCVs, although a contribution of synaptic-like microvesicle exocytosis cannot be excluded (22).

Rat β -cells release 25% of their GABA content per hour independently of the glucose concentration (38). This cannot be explained by release of GABA solely by exocytosis of LDCVs. Indeed, in cell clusters overexpressing GABA_AR, SR-95531 blocked a component of the holding current at 1 mmol/l glucose, indicating extracellular accumulation of GABA in the absence of detectable vesicular GABA release (23). Here we demonstrate that this also occurs in human β -cells. These data suggest that in addition to the glucose-dependent vesicular route of GABA release, a tonic glucose-independent background release mechanism exists in β -cells. Analysis of the excess noise (blockable by SR-95531) suggests that background release of GABA is only sufficient to activate individual channels. This indicates that background release of GABA is of a nonvesicular nature, the details of which remain to be elucidated but may involve GABA transporters operating in reverse or other nonvesicular routes (39).

Patch-clamp experiments revealed that human δ -cells express high levels of GABA_AR and that activation of these receptors—similar to what was observed in β -cells—depolarizes δ -cell membrane potential. In line with an excitatory effect of GABA_AR activation, blocking GABA_AR

reduced somatostatin secretion at 3 and 20 mmol/l but not at 10 mmol/l glucose. In agreement with the study of Xu et al. (8), the GABA_AR antagonist SR95531 also modulated glucagon secretion from human islets. The fact that so few isolated α -cells exhibited GABA-activated currents may suggest that the effects on glucagon secretion are principally mediated by paracrine effects. It should be noted, however, that 14% of the α -cells in intact islets contained detectable α_{1-6} subunit immunoreactivity. This is similar to the 16% obtained by electrophysiological analysis of α -cells preincubated in the presence of insulin and only slightly lower than the 22% observed in β -cells. Thus, it is possible that the expression of GABA_ARs in isolated α -cells is artifactually low because of the loss of intra-islet insulin signaling. In control experiments, we did not find any evidence that SR-95531 interferes nonspecifically with voltage-gated channels and electrical activity in islet cells. More work is needed to explain the effects of SR-95531 on glucagon and somatostatin secretion.

In summary, our data suggest that signaling via GABA and GABA_AR stimulates insulin secretion by a positive autocrine feedback loop in human β -cells. Many important pharmacological agents target GABA_AR, and it is possible that they, given the presence of GABA_AR in β -cells, also affect insulin secretion from pancreatic islets. Indeed, treatment of epileptic patients with the anticonvulsant valproic acid (which blocks the degradation of GABA) has been reported to result in increased postprandial insulin levels (40).

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