Insulin secretion stimulated by L-arginine and its metabolite L-ornithine depends on \( \Gamma_0_{12} \)

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Leiss V, Flockerzie K, Novakovic A, Rath M, Schönriegel A, Birnbaumer L, Schürmann A, Harteneck C, Nürnberg B. Insulin secretion stimulated by L-arginine and its metabolite L-ornithine depends on \( \Gamma_0_{12} \). Am J Physiol Endocrinol Metab 307: E800–E812, 2014. First published September 9, 2014; doi:10.1152/ajpendo.00337.2014.—Bor-detella pertussis toxin (PTx), also known as islet-activating protein, induces insulin secretion by ADP-ribosylation of inhibitory G proteins. PTx-induced insulin secretion may result either from inactivation of \( \Gamma_0 \) proteins or from combined inactivation of \( \Gamma_0_{1, \Gamma_0_{11}, \Gamma_0_{12}}, \) and \( \Gamma_0_{13} \) isoforms. However, the specific role of \( \Gamma_0_{12} \) in pancreatic \( \beta \)-cells still remains unknown. In global (\( \Gamma_0_{12} \)-/-) and \( \beta \)-cell-specific (\( \Gamma_0_{12} \)-/-) gene-targeted \( \Gamma_0_{12} \) mouse models, we studied glucose homeostasis and islet functions. Insulin secretion experiments and intracellular Ca\(^{2+} \) measurements were used to characterize \( \Gamma_0_{12} \) function in vitro. \( \Gamma_0_{12} \)-/- and \( \Gamma_0_{12} \)-/- mice showed an unexpected metabolic phenotype, i.e., significantly lower plasma insulin levels upon intraperitoneal glucose challenge in \( \Gamma_0_{12} \)-/- and \( \Gamma_0_{12} \)-/- mice, whereas plasma glucose concentrations were unchanged in \( \Gamma_0_{12} \)-/- but significantly increased in \( \Gamma_0_{12} \)-/- mice. These findings indicate a novel albeit unexpected role for \( \Gamma_0_{12} \) in the expression, turnover, and/or release of insulin from islets. Detection of insulin secretion in isolated islets did not show differences in response to high (16 mM) glucose concentrations between control and \( \beta \)-cell-specific \( \Gamma_0_{12} \)-/- mice. In contrast, the two- to threefold increase in insulin secretion evoked by L-arginine or L-ornithine (in the presence of 16 mM glucose) was significantly reduced in islets lacking \( \Gamma_0_{12} \). In accord with a reduced level of insulin secretion, intracellular calcium concentrations induced by the agonistic amino acid L-arginine \( \beta \)-cell did not reach control levels in \( \beta \)-cells. The presented analysis of gene-targeted mice provides novel insights in the role of \( \beta \)-cell \( \Gamma_0_{12} \) showing that amino acid-induced insulin-release depends on \( \Gamma_0_{12} \).

\( \Gamma_0_{12} \): insulin secretion; \( \beta \)-cell; L-arginine; GPCR

GLUCOSE IS THE PRINCIPAL STIMULATOR of insulin secretion from pancreatic \( \beta \)-cells. Together with regulators such as other nutrients or hormones, it adjusts insulin secretion according to physiological demands. A disruption of this tightly controlled process can lead to diabetes mellitus and its comorbidities. Physiological regulators of insulin release include not only glucose but also other nutrients such as free fatty acids or amino acids on the one hand, and hormones including glucagon and norepinephrine on the other hand. They all have in common that they signal via seven-transmembrane G protein-coupled receptors (GPCR) present on the surface of pancreatic \( \beta \)-cells (1, 28, 33). Upon ligand binding, GPCRs activate heterotrimeric G proteins, thereby modulating the activity of cellular effectors. It is widely accepted that insulin release from pancreatic \( \beta \)-cells can be triggered via \( \Gamma_0_{1-2} \) and/or \( \Gamma_0_{3} / \Gamma_0_{11} \)-dependent mechanisms (2, 41). Studies in isolated systems and in animals, using pertussis toxin (PTx), also known as islet-activating protein, suggest \( \Gamma_0_{1-2} / \Gamma_0_{11} \)-dependent signaling as being important for inhibition of insulin secretion (11, 14, 15). PTx specifically catalyzes the ADP-ribosylation of a cysteine residue located four residues from the carboxyl terminus of the \( \alpha \)-subunits of \( \Gamma_0_{1-2} / \Gamma_0_{11} \) proteins. Thus, PTx treatment disrupts GPCR stimulation of the G protein and thereby disconnects it from signal transduction. The \( \alpha \)-subunits of the PTx-sensitive G proteins include three \( \Gamma_0_{1} \) (\( \Gamma_0_{11}, \Gamma_0_{12}, \) and \( \Gamma_0_{13} \)) isoforms, which are the product of three different genes, and two \( \Gamma_0_{1} \) splice variants i.e., \( \Gamma_0_{11}, \Gamma_0_{12}, \) and \( \Gamma_0_{13} \) proteins, which show a sequence identity of up to 95% with overall sequence homology being more pronounced among the three \( \Gamma_0_{1} \) isoforms (37). Due to this high homology, it has been suggested that all \( \Gamma_0_{1} \) and \( \Gamma_0_{13} \) proteins serve the same functions; namely, they are activated by the same or a similar set of GPCRs and appear to signal to a fully overlapping set of effectors. However, combined analysis of global and islet cell-specific \( \Gamma_0_{11}, \Gamma_0_{12}, \) and/or \( \Gamma_0_{13} \)-deficient mouse lines and PTx treatment identified \( \Gamma_0_{12} \) as the main inhibitory G protein related to insulin release (32, 34, 42). Glucose-induced insulin secretion (34) and insulin containing vesicle docking (42) were increased in the absence of \( \Gamma_0_{12} \). Moreover, PTx treatment of \( \Gamma_0_{12} \)-deficient mice had a negligible effect on the already disarranged insulin secretion (42). These findings indicate that \( \Gamma_0_{12} \) proteins are the major isoform responsible for PTx-sensitive inhibition of insulin secretion. Additional evidence suggests that \( \Gamma_0_{12} \) mediates the somatostatin (SST)- and galanin-induced receptor-to-effec
tor signal transduction in \( \beta \)-cells (32). Together, these findings support a prominent role for \( \Gamma_0_{12} \) as the major regulator in GPCR-induced inhibition of insulin secretion. It is presently largely unclear whether the PTx-sensitive \( \Gamma_0_{12} \) isoform expressed in islets or elsewhere, such as intestine, skeletal muscle, or adipose tissue is of any significance for insulin secretion in vitro and/or glucose homeostasis in vivo. To elucidate the biological role and gain insight into the cellular mechanisms of...
Gα2 protein function in pancreatic β-cells, we examined global and β-cell-specific Gα2-deficient mice. We show that Gα2 mediates L-arginine-induced insulin secretion by modulation of corresponding changes in [Ca^{2+}]_i that trigger exocytosis. Decreased plasma insulin levels in Gα2-targeted animals leading to an impaired glucose tolerance supported these in vitro findings. These data strongly promote and unexpectedly stress a stimulatory role for Gα2 in insulin secretion, showing that although PTx inhibits Gαo and Gα2 proteins these two isoforms have distinct and opposite roles in the regulation of insulin secretion. Whereas Gαo inhibits, Gα2 stimulates insulin secretion from β-cells.

MATERIALS AND METHODS

Experimental animals. The generation of the global Gα2-deficient mice (Gα2−/−) has been described previously (29). To prolong life expectancy of the Gα2−/− mice, mice were bred and kept in individually ventilated cages (IVC) under specific-pathogen-free conditions (39) and had free access to water and standard chow. β-Cell-specific deletion of Gα2 (Gα2Δlck) was achieved by crossing the floxed Gα2 mouse line (27) and the Rip-Cre mouse line (27) and the Rip-Cre (tg) mouse line (12), both on a C57BL/6N background. The Rip-Cre+tg mouse line was a kind gift from Pedro Luis Herrera, Geneva. GαoΔlck and their littermate controls (ctl) were kept under specific-pathogen-free conditions with free access to water and standard chow. For all experiments, gene-targeted Gα2 animals were compared with their littermate wild-type (WT) or controls (genotype: Gα2+/−; Rip-Cre−/− or Gα2−/−; Rip-Cre−/−) on a C57BL/6N background. A second coat of hematoxylin (1% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin) were used and incubated in Krebs-buffered HEPES saline (120 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1 mM KH2PO4, 1.2 mM MgSO4, 10 mM HEPES, 20 mM NaHCO3, 0.5 mg/ml BSA, pH 7.4) containing 3 mM glucose in humidified 5% CO2 at 37°C for 30 min. Subsequently, the supernatant was discarded, and fresh Krebs-buffered HEPES saline with added supplemented with either 3 or 16 mM glucose with or without l-arginine (10 mM), l-ornithine (10 mM), l-leucine (100 μM), palmitate (0.2 mM), choline (1 μM), SST (0.1 μM), or KCl (30 mM). The islets were incubated in humidified 5% CO2 at 37°C for 1 h. Afterward, islets were treated with ice-cold acid ethanol (1.5% HCl, 70% ethanol) for 1 h. For each condition, aforementioned above 3 and 16 mM glucose was used to test the quality of islet preparation.

The amount of secreted insulin in the supernatant as well as whole insulin content was determined with a commercially available ultrasensitive insulin ELISA method (DRG Diagnostics, Marburg, Germany). Islet DNA was isolated with the IsoLate II genomic DNA kit (Bioline, Luckenwalde, Germany), and the islet DNA content was measured (30). Based on the identical DNA and whole insulin contents of Gα2Δlck and ctrl islets, the amounts of secreted insulin were indicated % of insulin content.

Immunoblot analysis. Freshly isolated islets were homogenized in 0.8 μl of protein lysis buffer per islet. The protein extractions were performed on pooled islets isolated from a single animal. To achieve electrophoretic separation of Gαo isoforms, separation was performed in gels containing 6 M urea. The proteins were visualized by immunodetection using the following primary antibodies described elsewhere (7, 10, 21, 39): rabbit anti-Gα2 (1:8,000 islets, 1:2,000 hypothalamus), rabbit anti-Gα2 (1:5,000), rabbit anti-Gα2 (1:2,000), and rabbit anti-Gβ2 common (1:9,000). Antibodies against Gαo(short) (1:1,000; SC-383) and Gα2 (1:1,000; SC-393) were purchased from Cell Signaling. Equal loading was verified with antibodies against mouse anti-α-tubulin (1:4,000, Sigma-Aldrich) or mouse anti-β-actin (1:4,000 islets, 1:40,000 hypothalamus; Sigma-Aldrich). The protein levels of Gα2, Gα3, Gαq, Gαo, Gβ1, and Gβ2 were quantified using densitometric analysis software (Image Lab; Bio-Rad, Gräfelfing, Germany) and were normalized to the α-tubulin or β-actin levels of the same samples.

Immunofluorescence. Immunodetection was performed on serial cryosections (8 μm) of pancreas fixed in 4% paraformaldehyde. For antigen retrieval, slices were treated with 0.3% Triton X-100-PBS for 30 min. To avoid unspecific antibody binding, slices were incubated in 5% normal goat serum (NGS)-PBS prior to incubations in primary antibody dilutions. Primary antibodies used were specific for rabbit anti-Gα2 (1:1,500), mouse anti-glucagon (1:5,000; Sigma-Aldrich, Hamburg, Germany), and guinea pig anti-insulin (1:5,000; DAKO, Hamburg, Germany) and diluted in 1.5% NGS-PBS. Binding of primary antibodies was performed at 4°C overnight. The complexes were detected with secondary antibodies conjugated to fluoresec dyes (1:200 dilution in PBS, Invitrogen). Slices were embedded in Roti-Mount-Fuoro-Dapi (Carl Roth, Karlsruhe, Germany). Fluorescence was visualized using either the Zeiss laser scanning microscope LSM 510 or the Zeiss Axio Image.M2 microscope (Zeiss, Jena, Germany).

Glucose tolerance tests. Mice were fasted overnight, followed by an intraperitoneal (ip) injection of glucose (2 mg/g body wt). Blood samples were collected via the tail vein, and blood glucose levels were measured using a Contour glucometer (Bayer, Leverkusen, Germany).

L-Ornithine test. Mice were fasted overnight but supplemented with 1 mM l-ornithine in their drinking water to ensure sufficient high basal l-ornithine levels in the plasma. Mice received 100 μl of a 1.32 mg/ml l-ornithine solution by ip injection, and blood samples were collected via tail vein before administration and after 1 min to determine plasma insulin levels with the commercially available ultrasensitive insulin ELISA method (see above).

Insulin tolerance test. Mice were fasted for 4 h, followed by an ip injection of insulin (1 μU/g body wt). Blood glucose levels were measured using a Contour glucometer (Bayer).

Plasma analysis. Plasma levels of insulin were measured using a commercially available insulin ELISA method (see above), whereas plasma C-peptide levels were determined with the 96-well fluorescent Milliplex immunoassay (Millipore, Darmstadt, Germany).
Ca\textsuperscript{2+} imaging. Intracellular calcium measurements were performed as previously described (16). Isolated islets were dispersed by trypsin-EDTA digestion, plated on glass coverslides, and allowed to recover for 4 h in humidified 5% CO\textsubscript{2} air at 37°C. Cells were loaded with Fura 2-AM (5μM) 30 min prior to calcium measurements. Changes of intracellular calcium concentrations [Ca\textsuperscript{2+}]\textsubscript{i} were recorded with a Till Photonics Oligochrome V (FEI, Gräfeling, Germany). After 4 min of baseline recordings in the presence of 5 mM glucose, dispersed islet cells were treated with 16 mM glucose, 10 mM L-arginine, 10 mM d-arginine, or a combination of 16 mM glucose and L-arginine for 10 min. Maximum depolarization of β-cells was evaluated by the use of 30 mM KCl. Emitted fluorescence was excited at 340 and 380 nm and measured at 520 nm, and the ratio F\textsubscript{380} / F\textsubscript{340} was calculated. Cells were identified as β-cells, when being inactive at 5 mM glucose, with stable baseline recordings, and an obvious response to the applied stimuli (e.g., 16 mM glucose, 10 mM L-arginine). Cells without Ca\textsuperscript{2+} responses to the respective stimuli were excluded from the measurements and analysis.

Statistical analysis. Data are presented as means ± SE of individual data points. Data were analyzed using Student’s t-test for unpaired groups. P < 0.05 was considered statistically significant.

RESULTS

Characterization of β-cell-specific G\textsubscript{0,12}-deficient mice.

The generation and characterization of global G\textsubscript{0,12}-deficient (G\textsubscript{0,12}\textsuperscript{-/-}) mice has been described (27, 29, 39). The β-cell-specific G\textsubscript{0,12}-deficient mice, termed G\textsubscript{0,12}\textsuperscript{bcko} (genotype: G\textsubscript{0,12}\textsuperscript{tnaRip-Cre}\textsuperscript{+/+}; Rip-Cre\textsuperscript{+tg}) or G\textsubscript{0,12}\textsuperscript{bcko} animals. Recombination was validated by PCR analysis of genomic DNA (Fig. 1, A and B). Whereas DNA isolated from hearts and islets from ctrl animals as well as cardiac DNA of the G\textsubscript{0,12}\textsuperscript{bcko} mice remained in the premutant state, islet DNA of G\textsubscript{0,12}\textsuperscript{bcko} mice showed clear recombination by appearance of an additional 390-bp band (Fig. 1, A and B). Since strong hypothalamic recombination has been reported in different Rip-Cre mouse lines (38), we analyzed DNA isolated from ctrl and G\textsubscript{0,12}\textsuperscript{bcko} hypothalamus. The absence of the 390-bp band indicative for recombination argues against significant recombination events in the hypothalamic brain region of our mouse line (Fig. 1B). The expression pattern of G\textsubscript{0,12} protein in pancreatic islets was studied by immunoblot analysis. Using specific antibodies against the COOH-terminal G\textsubscript{0,12} protein was absent in hypothalamic protein extracts from G\textsubscript{0,12}\textsuperscript{-/-} mice (Fig. 1E), whereas the G\textsubscript{0,12} protein levels of G\textsubscript{0,12}\textsuperscript{bcko} were unaffected, i.e., at control levels (Fig. 1, E and F). The combined analysis of G\textsubscript{0,12}\textsuperscript{bcko} tissues by genomic PCR and immunoblot make it very unlikely that the studied physiological effects resulted from cells other than the pancreatic β-cells.

Examination of Rip-Cre-mediated recombination was performed by staining of G\textsubscript{0,12} protein in pancreatic cryosections on the cellular level. β-Cells were visualized by insulin-specific antibodies (Fig. 1G). A diffuse yellow background staining was detectable; however, the specific G\textsubscript{0,12} staining was restricted in proximity to the plasma membrane. Insulin (blue) was found mainly in the cytoplasm in ctrl, G\textsubscript{0,12}\textsuperscript{bcko}, and G\textsubscript{0,12}\textsuperscript{-/-} islets (left). In ctrl islets, G\textsubscript{0,12} staining mainly localized to the plasma membrane (top middle) and was detectable in insulin-positive and insulin-negative cells (top right). In contrast, only a few primarily outer cells of the G\textsubscript{0,12}\textsuperscript{bcko} islets showed the specific membrane-close G\textsubscript{0,12} staining (middle, arrows). These G\textsubscript{0,12}-positive cells were all identified as insulin negative and therefore non-β-cells (middle right). In G\textsubscript{0,12}\textsuperscript{-/-} pancreata, the membrane-close G\textsubscript{0,12} staining was absent in all islet cells, insulin-negative and insulin-positive ones (bottom middle and bottom right). These results validated that G\textsubscript{0,12} and insulin could be found only in ctrl β-cells, whereas G\textsubscript{0,12}\textsuperscript{bcko} β-cells lacked the G\textsubscript{0,12} protein. Therefore, a successful recombination in G\textsubscript{0,12}\textsuperscript{bcko} islets was restricted to β-cells and demonstrated on a cellular level.

Analysis of G protein subunit levels in G\textsubscript{0,12}\textsuperscript{-/-} and G\textsubscript{0,12}\textsuperscript{bcko}

islets of Langerhans. Compensatory upregulation of closely related proteins is a threat of many studies using gene-deficient animals. The expression of almost all G\textsubscript{0,12} isoforms in pancreatic islets as well as G\textsubscript{0,q} and G\textsubscript{0,12} has been described. All of them perform major functions in the insulin secretion machinery. To get insight into possible upregulation and compensation in G\textsubscript{0,12} deficiency by other G protein family members, G\textsubscript{0,12}\textsuperscript{-/-} (Fig. 2) and G\textsubscript{0,12}\textsuperscript{bcko} (Fig. 3) islet homogenates were immunoblotted for putative changes in the expression of G\textsubscript{0,13}, G\textsubscript{0,13}G\textsubscript{0,12}G\textsubscript{0,q}, G\textsubscript{0,12}G\textsubscript{0,q}G\textsubscript{0,12}(short), and G\textsubscript{0,12}G\textsubscript{0,q}G\textsubscript{0,12}(long). The expression levels of the G\textsubscript{0,12}G\textsubscript{0,12}G\textsubscript{0,q} and G\textsubscript{0,12} isoforms remained at the control values in G\textsubscript{0,12}\textsuperscript{-/-} (Fig. 2, A–C) and G\textsubscript{0,12}\textsuperscript{bcko} islets (Fig. 3, A–C). In contrast, the highly homologous isoform G\textsubscript{0,13} was significantly upregulated in G\textsubscript{0,12}\textsuperscript{-/-} islets (Fig. 2D) (146.3 ± 14.3% of wt, P < 0.001). Importantly, in G\textsubscript{0,12}\textsuperscript{bcko} islets, G\textsubscript{0,13} protein levels remained unchanged (119.7 ± 12.2% of control, P = 0.22; Fig. 3D).

G proteins are heterotrimeric proteins consisting of an α-subunit and a βγ-complex. Upon ligand activation of a GPCR, the α-subunit dissociates from the receptor and from the βγ-complex. Both the α-subunit and the βγ-complex modulate the activity of a variety of effectors, including ion channels and enzymes. Due to high G\textsubscript{0} expression levels, the receptor-dependent activation of G\textsubscript{0} results in the release of significant amounts of βγ-complexes (4). Moreover, it has been shown that stabilization of GDP-bound Go by GBP is likely a major mechanism for the maintenance of stoichiometry between Go and GBP (9, 18). Therefore, an important point was to investigate whether expression levels of the β-isofoms were altered in G\textsubscript{0,12}\textsuperscript{-/-} and G\textsubscript{0,12}\textsuperscript{bcko} islets. Unaltered Gβ-protein levels, but simultaneously reduced Gα protein levels, might result in permanently free βγ-complexes and increased βγ-mediated effects. This could mask the physiological consequences of G\textsubscript{0,12} deletion. At least five different Gβ subunits (Gβ1–Gβ5)
have been described (13), with $\beta_1$ and $\beta_2$ being predominantly and ubiquitously expressed. In both mutant mouse lines, lower expression of $G_{\beta_1}$ ($G_{\alpha_2^{-/-}}$ 64.3 ± 9.6% of wt, $P = 0.06$; $G_{\alpha_2^{cko}}$ 61.1 ± 19.5% of ctrl, $P = 0.1$) and $G_{\beta_2}$ ($G_{\alpha_2^{-/-}}$ 80.3 ± 4.3% of wt, $P < 0.01$; $G_{\alpha_2^{cko}}$ 58.2 ± 8.2% of ctrl, $P < 0.01$) were noted in islet homogenates compared with the respective controls (Figs. 2E and 3E). However, the reduction of $G_{\beta_2}$ levels was more prominent in $G_{\alpha_2^{cko}}$ islets than in $G_{\alpha_2^{-/-}}$ islets, most likely due to increased $G_{\beta_2}$ protein levels (145%) in $G_{\alpha_2^{-/-}}$ islets. The reduced $G_{\beta}$ protein levels let it seem rather unlikely that effects observed in the gene-targeted mice resulted from increased $G_{\beta_2}\gamma$ complex activity.

**Metabolic evaluation of $G_{\alpha_2^{-/-}}$ and $G_{\alpha_2^{cko}}$ mice.** To study the consequences of $G_{\alpha_2}$ deletion on the metabolism, we performed metabolic analysis of WT vs. $G_{\alpha_2^{-/-}}$ (Fig. 4) and ctrl vs. $G_{\alpha_2^{cko}}$ (Fig. 5) mice. Global $G_{\alpha_2}$ deficiency is associated with a reduced body weight gain (22, 29), which was still observed when animals were kept under specific pathogen-free conditions (SPF) in IVCs (Fig. 4A). Differences in ad libitum-fed blood glucose levels were not obvious (Fig. 4B). Next an ip glucose tolerance test after overnight fasting was performed to analyze the impact of $G_{\alpha_2}$ deletion on the glucose homeostasis of these animals. Global deletion of $G_{\alpha_2}$ did not affect blood glucose levels during the glucose challenge
significantly increased blood glucose levels in the G\textsubscript{i2}\textsuperscript{cko} mice compared with WT mice (Fig. 4D). In addition, G\textsubscript{i2}\textsuperscript{-/-} animals displayed an improvement in insulin tolerance (Fig. 4E). To assess whether \(\beta\)-cell-specific deletion of G\textsubscript{i2} influences the body weight of G\textsubscript{i2}\textsuperscript{cko} mice, we recorded body weight and continuous measurements did not reveal any differences between the two groups (Fig. 5). Interestingly, significantly lower insulin levels were found in G\textsubscript{i2}\textsuperscript{cko} mice vs. the littermate controls (Fig. 5F). Consistent with the reduced plasma insulin, plasma C-peptide levels were significantly decreased at 15 and 30 min after the glucose bolus in G\textsubscript{i2}\textsuperscript{cko} mice (Fig. 5E). Insulin sensitivity, tested by the injection of insulin to 4-h-fasted G\textsubscript{i2}\textsuperscript{cko} and ctrl mice, revealed no differences between the two groups (Fig. 5F). These findings indicate that G\textsubscript{i2} is involved in the expression, turnover, or release of insulin from pancreatic islets. Higher plasma insulin levels would have been expected from previous PTx-based studies. Surprisingly, G\textsubscript{i2} deletion restricted to \(\beta\)-cells lowered plasma insulin levels. Collectively, these results indicate that deletion of G\textsubscript{i2} in \(\beta\)-cells impairs glucose tolerance by decreasing insulin secretion, suggesting that G\textsubscript{i2} is a positive regulator of insulin secretion.

**Islet function in vitro.** In G\textsubscript{i2}\textsuperscript{cko} mice, G\textsubscript{i2} deletion is restricted to \(\beta\)-cells, G\textsubscript{i3} is not upregulated in this conditional gene-targeted mouse line, and body weight is not affected by G\textsubscript{i2} deletion. Thus, these mice are an appropriate model for studying the molecular mechanism of G\textsubscript{i2} signaling in isolated islets independently of systemic effects. No abnormalities were detected in morphometric analysis of G\textsubscript{i2}\textsuperscript{cko} islets. Islet area, number of nuclei and islet DNA content were indistinguishable between ctrl and G\textsubscript{i2}\textsuperscript{cko} islets (Fig. 6, F–H). We performed insulin secretion studies on islets isolated from G\textsubscript{i2}\textsuperscript{cko} and ctrl mice. Insulin release was studied either
under low (3 mM) or high (16 mM) glucose concentrations. All \( \alpha_i \) and \( \beta \)-subunit expression levels are not influenced by \( \alpha_i \)-deletion in \( \alpha_i \)-islets. \( \alpha_i \) antibody specificity was shown by loading of \( \alpha_i \)-islets. E: downregulation of \( \beta \)- and \( \delta \)-expression levels in \( \alpha_i \)-islets. \( \delta \)-downregulation gains statistical significance. Insets: representative immunoblots of islet homogenates from wt and \( \alpha_i \)-or ctrl and \( \alpha_i \)-animals. Equal loading was confirmed by \( \beta \)-actin detection.

**Fig. 3.** Statistical analysis of different \( \alpha_i \) and \( \beta \)-subunit expression patterns in \( \alpha_i \)-islets. \( \alpha_i \), \( \alpha_i \)-long, \( \alpha_i \)-short, \( \alpha_i \)-q, and \( \alpha_i \)-i protein expression levels are not influenced by \( \alpha_i \)-deletion in \( \alpha_i \)-islets. \( \alpha_i \) antibody specificity was shown by loading of \( \alpha_i \)-islets. E: downregulation of \( \beta \)-expression levels in \( \alpha_i \)-islets. \( \delta \)-downregulation gains statistical significance. Insets: representative immunoblots of islet homogenates from wt and \( \alpha_i \)-or ctrl and \( \alpha_i \)-animals. Equal loading was confirmed by \( \beta \)-actin detection.

under low (3 mM) or high (16 mM) glucose concentrations. All \( \alpha_i \)- and \( \beta \)-islet produced a robust insulin-secretory response to high glucose concentrations, whereas insulin release was almost absent at low glucose concentrations (Fig. 7A and Table 1). Insulin secretion evoked by high \( K^+ \) concentration, which bypasses glucose metabolism, was also not affected by the deletion of \( \alpha_i \) in \( \alpha_i \)-islets (Fig. 7A). Since a large body of PTx-based studies suggested that \( \alpha_i \) proteins inhibit insulin secretion (11, 15, 28), we tested clonidine- and somatostatin (SST)-induced inhibition of insulin secretion. The inhibiting effects of clonidine (1 \( \mu \)M) and SST (100 nM) were unaffected by the absence of \( \alpha_i \) in \( \alpha_i \)-islets (Figs. 7, B and C). This argues against an inhibitory role of \( \alpha_i \) in the regulation of insulin secretion from \( \beta \)-cells. In contrast, significantly reduced in vivo plasma insulin levels in \( \alpha_i \)-islets suggest that \( \alpha_i \) modulates glucose-induced insulin release by an unknown parameter, for instance, a factor present in the serum, i.e., fatty acids and/or amino acids, which signals via \( \beta \)-actin GPCRs (37). To identify the stimulus, we analyzed the effect of the free fatty acid palmitate on insulin secretion in \( \alpha_i \)-islets. Simultaneous stimulation of insulin with high glucose and palmitate (200 \( \mu \)M) resulted in increased insulin secretion in \( \alpha_i \)-islets (Fig. 8A). Next, we studied amino acids. Application of L-leucine (100 \( \mu \)M) did not alter the insulin response either in ctrl or \( \alpha_i \)-islets (Fig. 8B). However, application of L-arginine and L-ornithine surprisingly produced significantly differing insulin secretion responses in ctrl and \( \alpha_i \)-islets. Costimulation of L-arginine (10 mM) together with glucose (16 mM) showed decreased insulin release from \( \alpha_i \)-islets compared with ctrl islets (Fig. 8C). To test whether L-arginine signals via nitric oxide (NO), we used L-ornithine. In combination with glucose, L-ornithine (10 mM) was able to mimic the effect observed with L-arginine (Fig. 8D). None of the tested inhibitors or stimulators used within this setup was able to induce significant insulin secretion from isolated islets in the presence of 3 mM glucose (Table 1). To test whether our findings of significantly reduced L-arginine- and L-ornithine-induced insulin secretion from \( \alpha_i \)-islets are of any physiological relevance, we performed an in vivo L-ornithine challenge. L-Ornithine was used instead of the more potent stimulus L-arginine to exclude interferences by NO-driven pathways. We studied whether a single dose of L-ornithine (1.32 \( \mu \)g/ml L-ornithine ip) affects plasma insulin levels in vivo in male mice. Whereas plasma
insulin levels of control mice immediately increased after the
L-ornithine application, plasma insulin levels of Goα2<sup>-/-</sup>
animals remained at basal levels (Fig. 8E). Taken together, the
reduced L-ornithine-induced insulin secretion in vitro and in
vivo strengthen the assumption that the PTx-sensitive Goα2
isofor is not an inhibitor but a stimulator of insulin secretion.

\[ [\text{Ca}^{2+}]_i \]. measurements in islets. To gain detailed insight into
the signaling pathway, we aimed to link our in vitro findings to
an intracellular correlate. It is commonly accepted that insulin
secretion is a result of an increase in [Ca<sup>2+</sup>]. This can be
determined in single islet cells and is therefore a cell-based
 correlate for β-cell insulin secretion. Measurements of intra-
cellular calcium changes link the Goα2-dependent signaling to
insulin secretion at a cellular level. Changes in [Ca<sup>2+</sup>], signals
for individual β-cells were recorded in Fura 2-loaded single
islet cells. For basal conditions, β-cells were kept at 5 mM
 glucose before treatment with 16 mM glucose. Control and
Goα2<sup>lcko</sup> single islet cells showed a similar increase in [Ca<sup>2+</sup>],
in response to the application of 16 mM glucose (Fig. 9A). The
Ca<sup>2+</sup> response was significantly reduced in Goα2<sup>lcko</sup> islet cells
when stimulated with L-arginine (10 mM) in the presence of
basal glucose levels (5 mM) (Fig. 9B). Being aware that a
simple depolarization event could be caused by the positive
charge of the basic amino acid, we tested D-arginine (10 mM),
the enantiomer of L-arginine. In contrast to L-arginine, D-argi-
nine did not induce a calcium response either in G<sup> wt</sup>
 islets, whereas the response of G<sup> i2<sup>-/-</sup> </sup>cells was even more
reduced response to L-arginine of G<sup> i2<sup>-/-</sup> </sup>cells was calculated as the difference between the
maxima obtained with glucose alone (Fig. 9C). This can be
explained by the fact that L-arginine is a more potent inducer of
Ca<sup>2+</sup> responses in islet cells compared to glucose (Fig. 9D). The
l-arginine-induced increase in [Ca<sup>2+</sup>], was calculated as the difference between the
maxima obtained with glucose plus l-arginine (Fig. 9D) minus
the maxima obtained with glucose alone (Fig. 9A). The co-
treatment resulted in a robust increase in [Ca<sup>2+</sup>], in control
islets, whereas the response of Goα2<sup>lcko</sup> islet cells was signif-

Fig. 4. Metabolic phenotyping of Goα2<sup>-/-</sup>
animals. A: body weight gain of wt and
Goα2<sup>-/-</sup> mice. Body weight of Goα2<sup>-/-</sup> mice is significantly reduced. B: blood glucose levels
determined in wt and Goα2<sup>-/-</sup> animals over time, which are comparable between the 2
genotypes (n = 16 animals per genotype). C: blood glucose levels in 8- to 10-wk-old wt (■)
and Goα2<sup>-/-</sup> (□) mice following ip application of glucose after overnight fasting are equal (n = 8 animals per group). D: Plasma insulin levels
during ip glucose tolerance testing in wt and Goα2<sup>-/-</sup> animals (n = 8 animals per
 group). Plasma insulin levels are significantly reduced at 15, 30, and 60 min. E: insulin
tolerance testing in 4-h-fasted wt and Goα2<sup>-/-</sup> mice (n = 6 per genotype). *P < 0.05.
The present study analyzed the physiological role of Go\textsubscript{i2} in insulin-secreting \( \beta \)-cells by using global and \( \beta \)-cell-specific Go\textsubscript{i2} mutants. Significantly lower plasma insulin levels were observed upon glucose challenge in both the Go\textsubscript{i2}\textsuperscript{cko} and the Go\textsubscript{i2}\textsuperscript{-/-} animal models. A normal glucose-induced insulin secretion in vitro per se accompanied these in vivo findings. Importantly, the selective and specific stimulatory effects of amino acids such as L-arginine and L-ornithine on insulin secretion were significantly diminished in Go\textsubscript{i2}-deficient \( \beta \)-cells. This was not due to alterations in islet morphology, since islet size, the number of nuclei per islet, and islet cell size were unaffected by the Go\textsubscript{i2} deficiency. This study provides causal evidence for the involvement of Go\textsubscript{i2} in the positive regulation of plasma insulin levels and glucose tolerance in vivo and islet insulin secretion in vitro. Although PTx-based studies in mice and isolated islets assumed an inhibitory role for all Go\textsubscript{i} and Go\textsubscript{\alpha} proteins, our data surprisingly indicate that Go\textsubscript{i2} proteins rather stimulate than inhibit the secretion of insulin by regulating L-arginine- and L-ornithine-induced insulin secretion in vitro and in vivo.

Currently, the upstream regulator of Go\textsubscript{i2} is unknown. Two recent studies describe mouse models lacking the nutrient receptor GPCR family C group 6 member A (GPRC6A), which is a receptor for L-amino acids (35), in particular those with basic side chains (24, 36). Whereas Smajilovic et al. (31) found evidence for the expression of GPRC6A in pancreatic islets but found no evidence that the L-arginine-induced insulin secretion was affected by GPRC6A deletion, Pi et al. (25, 26) reported that GPRC6A mediates osteocalcin- and L-arginine-induced insulin secretion from \( \beta \)-cells in global and \( \beta \)-cell-specific GPRC6A-deficient mouse models. Our data from global and
β-cell-specific Gα12-deficient mice showing significantly decreased plasma insulin levels following glucose challenge are consistent with those from global and β-cell-deficient GPRC6A mice (25). Future studies will have to clarify whether the concordant findings in the GPRC6A-deficient and Gα12-deficient mice are mechanistically connected.

Gα12-deficient islets secreted significantly less insulin upon co-stimulation with glucose and L-ornithine or L-arginine. These findings are complementary to recent studies analyzing global and β-cell-specific gene-targeted Gαi mice (42). Those authors suggest that Gαi is a strong inhibitor of insulin secretion, since its ablation produced a strong stimulatory effect on insulin release. Gαi has been implicated in regulating insulin vesicle docking on β-cell membranes (42) and in inhibiting insulin release via signaling through galanin- and SST-activated GPCRs (32, 34). In line with these findings, herein we find significant inhibition of insulin secretion by SST in both control and Gα12-deficient islets. Of course, we are aware of the fact that these findings conflict with the expected functions for Gαi based on PTx experiments, a pan inhibitor of Gαi and Gαo proteins and therefore a strong stimulator of insulin secretion. However, clonidine, an α2-adrenergic receptor agonist, completely inhibited

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**Fig. 6.** Morphometric analyses of Gα12-deficient islets. A: representative H&E-stained ctrl and Gα12-deficient islets. B: representative sections of pancreata from ctrl and Gα12-deficient mice visualized by immunofluorescence with anti-insulin (red) and anti-glucagon (green) antibodies. Scale bar, 40 μm. Islet size (C), number of nuclei per islet (D), and mean cell size (E) of ctrl and Gα12-deficient islets were equal. Morphometric analyses were performed in 6-wk-old male mice. In total, 381 control and 455 Gα12-deficient islets derived from 3 mice per genotype were quantified for this evaluation. Whole pancreas insulin content (F), islet insulin (G), and islet DNA content (H) of ctrl and Gα12-deficient mice did not differ between genotypes. Islet insulin or islet DNA content was determined following static incubation experiments (n, number of triplicates).
insulin release in control and Go12cko islets. Thus, our data set provides additional evidence that Go12 is not involved in the α2-adrenoreceptor-mediated signaling. The inhibition of the inhibitory Go12-driven pathway by PTx may be the major cause of PTx-induced insulin release from islets.

Moreover, the PTx effect was negligible in β-cell-specific Go12-deficient animals (42). This suggests that, despite the fact that Go1 proteins being expressed in islet cells and ADP-riboseylated by PTX, Go1 is the major target for PTx stimulating insulin secretion, thereby making an inhibitory role of Go12 very unlikely (42). A single study shows an inhibitory role for Go12 in ghrelin-dependent inhibition of insulin secretion based on an antisense oligonucleotide approach (5). Our data demonstrate that deletion of Go12 resulted in normal glucose-induced insulin secretion per se, but stimulatory effects of amino acids like L-arginine and L-ornithine on insulin secretion were impaired in Go12-deficient islets. Moreover, Go12cko mice exhibit impaired glucose tolerance caused by significantly decreased plasma insulin levels. This is also in contrast to data obtained from PTx-based studies, strengthening again the fact that Go12 is an important stimulator of insulin secretion.

This view is also supported by the failure of in vivo-applied L-ornithine to provoke a rise in insulin plasma levels in β-cell-specific Go12-deficient animals, which was seen in littermate controls.

Our detailed analysis of G protein levels in the Go12 gene-targeted mouse lines corroborates significant upregulation of Go13 (about 45%) in the global Go12-deficient mice, which has also been described for other tissues and organs (6, 17, 39, 40). In contrast to the global Go12-deficient islets, the Go13 protein levels were unchanged in Go12cko islets. Since plasma insulin levels are reduced in both gene-targeted mouse lines, Go13 is obviously not able to compensate for the lack of Go12. This confirms previous reports on specific and distinct functions of Go12 and Go13 (8, 10, 17). Reduced Gβ expression levels parallel deletion of Go12. Heterotrimeric G proteins are stoichiometrically composed of Gα and Gβγ, which dissociate selectively during the activation cycle. The stabilization of Gα and Gβγ by the trimeric complex is likely a major mechanism for the maintenance of the stoichiometric equivalence between Gα and Gβγ in the cell (9, 18). We found Gβ levels significantly reduced in both global and β-cell-specific Go12-deficient

### Table 1. Insulin secretion evoked by 3 mM glucose concentration and in the presence of the respective substances

<table>
<thead>
<tr>
<th>Condition</th>
<th>Secreted insulin content</th>
<th>n</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%Insulin content SE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 mM glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 mM glucose</td>
<td>0.189 ± 0.02</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>1 μM clonidine</td>
<td>0.153 ± 0.05</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>100 nM SST</td>
<td>0.176 ± 0.05</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>200 mM palmitate</td>
<td>0.324 ± 0.04</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>100 μM L-leucine</td>
<td>0.292 ± 0.05</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>10 mM L-arginine</td>
<td>0.075 ± 0.02</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>10 mM L-ornithine</td>
<td>0.150 ± 0.02</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>10 mM clonidine</td>
<td>0.153 ± 0.05</td>
<td>5</td>
<td>n.s.</td>
</tr>
<tr>
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<td>0.150 ± 0.02</td>
<td>6</td>
<td>n.s.</td>
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n.s., not significant; n, set of triplicates.
mouse lines, with a more pronounced reduction of Gβ levels in Gα2-/-/β2-deficient mice (see Fig. 3E) than in Gα2-/-/β2-deficient islets (see Fig. 2E). It is reasonable to assume that the differences in the extent of reduction between Gα2-/-/β2-deficient and Gα2-/-/β2-deficient islets result from the selective upregulation of Gα3 protein in Gα2-/-/β2-deficient animals only, as Gα3 also requires Gβ as counterpart for the stabilization. This is in line with results from approaches using shRNA to downregulate Gβ isoforms in HeLa cells, which was accompanied with parallel reduced Gα protein levels in this cell line. Vice versa, several studies show that shRNA against Gα2 resulted in decreased Gβ1, Gβ2, and Gβ3 protein levels (18, 39, 40). It has to be noted that Gβ3 is the predominant isoform in islets, which has been reported only for human placenta (23). Signaling by the Gαi family of “inhibitory” proteins is complex. Gαi proteins can activate or inhibit many other effectors through either the GTP-bound Gαi or the released Gβγ dimer upon activation. Therefore, it is important to note that both the abolished signaling of Gα2 via direct interaction partners and the signaling via Gβγ complexes may equally contribute to the observed phenotypes of our gene-targeted mice.

The reduced insulin levels during glucose tolerance tests were observed in global and β-cell-specific Gα2-deficient mice. Differences in glucose were visible only in Gα2-/-/β2-deficient animals, not in mice globally lacking Gα2. Wang et al. (34) also analyzed blood glucose levels of global Gα2-deficient mice, which were similar to our studies, unaltered upon glucose challenge. However, those authors did not study β-cell-specific Gα2 mutants, and they did not report the plasma insulin levels during the glucose challenge. A reduced body weight of Gα2-/- mice has been reported (29) and is also observed in our mouse line. A lean phenotype improves glucose tolerance per se, which might explain similar blood glucose levels but simultaneously decreased plasma insulin levels in global Gα2-deficient mice. In line with this, Gα2-deficient mice showed improved insulin tolerance, arguing for the improved peripheral insulin sensitivity. The lean phenotype was not observed in β-cell-specific Gα2 mutants. Control and mutant animals showed similar weight gain. However, Gα2-/-/β2-deficient animals showed impaired glucose tolerance caused by impairment of the β-cells’ ability to secrete the required amount of insulin. The differences in

Fig. 8. Effect of different stimuli on insulin secretion experiments in ctrl and Gα2-/-/β2-deficient islets. Stimulatory effect of palmitic acid (A), and l-leucine (B) was not affected by deletion of Gα2 in β-cells. Potentiating effect of l-arginine (C) and L-ornithine (D) on insulin secretion was significantly reduced in Gα2-/-/β2-deficient islets (see Fig. 3E) than in Gα2-/-/β2-deficient islets (see Fig. 2E). It is reasonable to assume that the differences in the extent of reduction between Gα2-/-/β2-deficient and Gα2-/-/β2-deficient islets result from the selective upregulation of Gα3 protein in Gα2-/-/β2-deficient animals only, as Gα3 also requires Gβ as counterpart for the stabilization. This is in line with results from approaches using shRNA to downregulate Gβ isoforms in HeLa cells, which was accompanied with parallel reduced Gα protein levels in this cell line. Vice versa, several studies show that shRNA against Gα2 resulted in decreased Gβ1, Gβ2, and Gβ3 protein levels (18, 39, 40). It has to be noted that Gβ3 is the predominant isoform in islets, which has been reported only for human placenta (23). Signaling by the Gαi family of “inhibitory” proteins is complex. Gαi proteins can activate or inhibit many other effectors through either the GTP-bound Gαi or the released Gβγ dimer upon activation. Therefore, it is important to note that both the abolished signaling of Gα2 via direct interaction partners and the signaling via Gβγ complexes may equally contribute to the observed phenotypes of our gene-targeted mice.

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blood glucose levels between global and β-cell-specific G\(_{\alpha\,i2}\) mice imply that the metabolic functions of G\(_{\alpha\,i2}\) in the body are broader than previously thought. G\(_{\alpha\,i2}\) signaling might play a role for glucose homeostasis in liver, white adipose tissue, or skeletal muscle as well, but G\(_{\alpha\,i2}\) signaling is also important for efficient pancreatic β-cell function. Thus, we propose that the diminished plasma insulin levels indeed result from islet G\(_{\alpha\,i2}\) dysfunction, caused by an impaired potentiating effect of L-arginine and L-ornithine in pancreatic β-cells. In addition, the reduced increases of intracellular calcium levels upon L-arginine treatment in G\(_{\alpha\,i2}^{\text{cko}}\)-β-cells compared with ctrl-β-cells, whereas the response is the difference between the maxima obtained with glucose plus L-arginine (D) minus the maxima obtained with glucose alone (A); n = 3–4 animals per group. All recordings were started at 5 mM glucose with additions as shown on the figure.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

REFERENCES

1. Ahren B. Islet G protein-coupled receptors as potential targets for treat-


5. Dezaki K, Kakei M, Yada T. Ghrelin uses G0i2 and activates voltage-
dependent K+ channels to attenuate glucose-induced Ca2+ signaling and
insulin release in islet β-cells: novel signal transduction of ghrelin.


7. Exner T, Jensen ON, Mann M, Kleeus C, Nürnberg B. Posttranslational modifica-

8. Ezhizhini E, Knaut K, Nürnberg B. The heterotrimeric G protein Gao32 regulates hepatic autophagy downstream of the insulin receptor. Au-


10. Glowinski J, Kleiber M, Nürnberg B. The dopamine G protein Gao32 regulates hepatic autophagy downstream of the insulin receptor. Au-


15. Katada T, Ui M. In vitro effects of islet-activating protein on cultured rat pancreas: enhancement of insulin secretion, adenose 3’-5’ mono-


18. Nürnberg B, Rambaum M, Tabachnick II. Species- and tissue-dependent diversity of G-protein β-subunit phosphory-


24. Schulz N, Kühf O, Jastroch M, Schürmann A. Minor role of mito-


35. Zawaliesz WS, Zawaliesz KC. Regulation of insulin secretion by phos-