Insulin secretion stimulated by L-arginine and its metabolite L-ornithine depends on Go\textsubscript{i2}

Veronika Leiss,1 Katarina Flockerzie,1 Ana Novakovic,1 Michaela Rath,2 Annika Schönziegel,1 Lutz Birnbaumer,3 Annette Schürmann,2 Christian Harteneck,1 and Bernd Nürnberg1

1Department of Pharmacology and Experimental Therapy, Institute of Experimental and Clinical Pharmacology and Toxicology, Eberhard Karls University Hospitals and Clinics, and Interfaculty Center of Pharmacogenomics and Drug Research, University of Tübingen, Tübingen, Germany; 2Department of Experimental Diabetology, German Institute of Human Nutrition, Potsdam-Rehbrücke, Nuthetal, Germany; 3Laboratory of Neurobiology, National Institute of Environmental Health Sciences, National Institutes of Health/Department of Health and Human Services, Durham, North Carolina

Submitted 16 July 2014; accepted in final form 28 August 2014

Leiss V, Flockerzie K, Novakovic A, Rath M, Schönziegel A, Birnbaumer L, Schürmann A, Harteneck C, Nürnberg B. Insulin secretion stimulated by L-arginine and its metabolite L-ornithine depends on Go\textsubscript{i2}. Am J Physiol Endocrinol Metab 307: E800–E812, 2014. First published September 9, 2014; doi:10.1152/ajpendo.00337.2014.—Bordetella 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 Go\textsubscript{o2} proteins or from combined inactivation of Go\textsubscript{i1}, Go\textsubscript{i2}, and Go\textsubscript{i3} isoforms. However, the specific role of Go\textsubscript{i2} in pancreatic β-cells still remains unknown. In global (Go\textsubscript{i2}\textsuperscript{−−}) and β-cell-specific (Go\textsubscript{i2}\textsuperscript{βς}βς) gene-targeted Go\textsubscript{i2} mouse models, we studied glucose homeostasis and islet functions. Insulin secretion experiments and intracellular Ca\textsuperscript{2+} measurements were used to characterize Go\textsubscript{i2} function in vitro. Go\textsubscript{i2}\textsuperscript{−/−} and Go\textsubscript{i2}\textsuperscript{βς/βς} mice showed an unexpected metabolic phenotype, i.e., significantly lowered plasma insulin levels upon intraperitoneal glucose challenge in Go\textsubscript{i2}\textsuperscript{−/−} and Go\textsubscript{i2}\textsuperscript{βς/βς} mice, whereas plasma glucose concentrations were unchanged in Go\textsubscript{i2}\textsuperscript{−/−} but significantly increased in Go\textsubscript{i2}\textsuperscript{βς/βς} mice. These findings indicate a novel albeit unexpected role for Go\textsubscript{i2} 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 Go\textsubscript{i2}\textsuperscript{−/−} cells still remains unknown. In accord with a reduced level of insulin secretion, intracellular calcium concentrations induced by the agonistic amino acid L-arginine did not reach control levels in β-cells. The presented analysis of gene-targeted mice provides novel insights in the role of β-cell Go\textsubscript{i2} showing that amino acid-induced insulin-release depends on Go\textsubscript{i2}.

Go\textsubscript{i2}: insulin secretion; β-cell; L-arginine; GPCR

Glucose is the principal stimulator of insulin secretion from pancreatic β-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 β-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 β-cells can be triggered via Go\textsubscript{o2} and/or Go\textsubscript{i1} dependent mechanisms (2, 41). Studies in isolated systems and in animals, using pertussis toxin (PTx), also known as islet-activating protein, suggest Go\textsubscript{i1}/Go\textsubscript{o1}-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 α-subunits of Go\textsubscript{o1}/Go\textsubscript{o2} proteins. Thus, PTx treatment disrupts GPCR stimulation of the G protein and thereby disconnects it from signal transduction. The α-subunits of the PTx-sensitive G proteins include three Go\textsubscript{o1} (Go\textsubscript{o1i}, Go\textsubscript{o2i}, and Go\textsubscript{o3i}) proteins, which are the product of three different genes, and two Go\textsubscript{i} splice variants i.e., Go\textsubscript{i1} and Go\textsubscript{i2} (3). Go\textsubscript{o1}/Go\textsubscript{o2} proteins show a sequence identity of up to 95% with overall sequence homology being more pronounced among the three Go\textsubscript{i} isoforms (37). Due to this high homology, it has been suggested that all Go\textsubscript{i} and Go\textsubscript{o} 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 Go\textsubscript{o1i} and/or Go\textsubscript{i2} deficient mouse lines and PTx treatment identified Go\textsubscript{o2} 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 Go\textsubscript{o2}. Moreover, PTx treatment of Go\textsubscript{o2} deficient mice had a negligible effect on the already disarranged insulin secretion (42). These findings indicate that Go\textsubscript{i} proteins are the major isoform responsible for PTx-sensitive inhibition of insulin secretion. Additional evidence suggests that Go\textsubscript{i2} mediates the somatostatin (SST)- and galanin-induced receptor-to-effector signal transduction in β-cells (32). Together, these findings support a prominent role for Go\textsubscript{i2} as the major regulator in GPCR-induced inhibition of insulin secretion. It is presently largely unclear whether the PTx-sensitive Go\textsubscript{i2} 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
G_{\alpha_2} protein function in pancreatic β-cells, we examined global and β-cell-specific G_{\alpha_2}-deficient mice. We show that G_{\alpha_2} mediates l-arginine-induced insulin secretion by modulation of corresponding changes in \([\text{Ca}^{2+}]_i\) that trigger exocytosis. Decreased plasma insulin levels in G_{\alpha_2}-targeted animals leading to an impaired glucose tolerance supported these in vitro findings. These data strongly promote and unexpectedly stress a stimulatory role for G_{\alpha_2} in insulin secretion, showing that although PTx inhibits G_{\alpha_1} and G_{\alpha_2} proteins these two isoforms have distinct and opposite roles in the regulation of insulin secretion. Whereas G_{\alpha_1} inhibits, G_{\alpha_2} stimulates insulin secretion from β-cells.

MATERIALS AND METHODS

Experimental animals. The generation of the global G_{\alpha_2}-deficient mice (G_{\alpha_2}^+/−) has been described previously (29). To prolong life expectancy of the G_{\alpha_2}^+/− +/− animals, mice were bred and kept individually ventilated caged (IVC) under specific-pathogen-free conditions (39) and had free access to water and standard chow. β-Cell-specific deletion of G_{\alpha_2} (G_{\alpha_2}^{cre}) was achieved by crossing the floxed G_{\alpha_2} mouse line (27) and the Rip-Cre +/−/+ mouse line (12), both on a C57BL/6N background. The Rip-Cre +/−/+ mouse line was a kind gift from Pedro Luis Herrera, Geneva. G_{\alpha_2}^{cre} 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 G_{\alpha_2} animals were compared with their littermate wild types (WT) or controls (genotype: G_{\alpha_2}^+/−; Rip-Cre +/−/+ or G_{\alpha_2}−/−; Rip-Cre +/−/+ on a C57BL/6N background (age 6–24 wk). All animals analyzed within this study were maintained and bred in the animal facility of the Institute of Experimental and Clinical Pharmacology and Toxicology, Eberhard Karls University, Tübingen. All experimental procedures were approved by the local government’s Committee on Animal Care and Welfare Tübingen.

Isolation of pancreatic islets. Islets were isolated according to procedure in Ref. 20. Upon a retrograde injection of 2 ml of collagenase solution (1.9 U/ml collagenase; Sigma Aldrich, Munich, Germany) in 1× HBSS (Life Technologies, Darmstadt, Germany) via the bile duct, fully perfused pancreata were incubated for 10 min at 37°C. Islets were picked using a 200-μl pipette during several washing steps.

Whole pancreatic insulin content. Pancreata were removed and weighed. After a homogenization step in 7 ml of ice-cold 1.5% HCl in 70% ethanol, pancreata were incubated in acid ethanol for 24 h at 4°C. Insulin content was determined in the supernatant with the commercially available ultrasensitive insulin ELISA method (DRG Diagnostics, Marburg, Germany). The protein levels of G_{\alpha_2}, G_{\alpha_2}^{s}, G_{\alpha_2}^{i}, G_{\alpha_2}^{q}, G_{\alpha_2}^{1}, and G_{\beta_2} were quantified using densitometric analysis software (Image Lab; Bio-Rad, Gräfelfing, 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 fluorescent dyes (1:200 dilution in PBS, Invitrogen). Slices were embedded in 3% paraformaldehyde 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 the 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 with a PicoGreen assay (Molecular Probes). The proteins were visualized by immunodetection using the following primary antibodies described elsewhere (7, 10, 21, 39): rabbit anti-G_{\alpha_2} (1:8,000 islets, 1:2,000 hypothalamus), rabbit anti-G_{\alpha_2} (1:5,000), rabbit anti-G_{\alpha_2} (1:2,000), and rabbit anti-G_{\beta_2} (1:9,000). Antibodies against G_{\alpha_2} (short/long) (1:1,000; SC-383) and G_{\alpha_2} (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 G_{\alpha_2}, G_{\alpha_3}, G_{\alpha_4}, G_{\beta_2}, and G_{\beta_2} were quantified using densitometric analysis software (Image Lab; Bio-Rad, Gräfelfing, Germany) and normalized to the α-tubulin or β-actin levels of the same samples.

Immunofluorescence. Immunodetection was performed on serial cryosections (8 μm) of pancreata 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 solutions. Primary antibodies used were specific for rabbit anti-G_{\alpha_2} (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 fluorescent dyes (1:200 dilution in PBS, Invitrogen). Slices were embedded in Roti-Mount-Fluoro-Dapi (Carl Roth, Karlsruhe, 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). 1-Ornithine test. Mice were fasted overnight but supplemented with 1 mM 1-ornithine in their drinking water to ensure sufficient high basal 1-ornithine levels in the plasma. Mice received 100 μl of a 1.32 mg/ml 1-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 Milliiplex immunoassay (Millipore, Darmstadt, Germany).
Ca\(^{2+}\) 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\(_2\) air at 37°C. Cells were loaded with Fura 2-AM (5 μM) 30 min prior to calcium measurements. Changes of intracellular calcium concentrations were recorded with a Till Photonics Oligochrome V (FEI, Gräfenhain, 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 t-arginine, 10 mM d-arginine, or a combination of 16 mM glucose and t-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 340/380 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 t-arginine). Cells without Ca\(^{2+}\) 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\(_{i2}\)-deficient mice. The generation and characterization of global G\(_{i2}\)-deficient (G\(_{i2}^{-/-}\)) mice has been described (27, 29, 39). The β-cell-specific G\(_{i2}\)-deficient mice, termed G\(_{i2}\)-B\(_{i2ko}\) (genotype: G\(_{i2}\)-/Rip-Cre+/-; R\(_{i2}\)-B\(_{i2ko}\)) were generated by crossing Rip-Cre mice (12) with mice carrying floxed G\(_{i2}\) alleles (27). The resulting progeny, i.e., the different genotypes, were born at expected Mendelian ratios and were viable and fertile. Since the expression of the Rip-Cre transgene has been reported to have metabolic effects per se (19), we used Rip-Cre-positive littermates throughout this study as controls (ctrl genotype: G\(_{i2}\)-+/; Rip-Cre+/- or G\(_{i2}\)-/Rip-Cre+/-) for experiments including G\(_{i2}\)-B\(_{i2ko}\) mice after having confirmed that heterozygous mice, e.g., G\(_{i2}\)-/Rip-Cre+/- or G\(_{i2}\)-+/Rip-Cre+/- mice were indistinguishable from G\(_{i2}\)-B\(_{i2ko}\)-/ and G\(_{i2}\)-B\(_{i2ko}\)-/+ 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\(_{i2}\)-B\(_{i2ko}\) mice remained in the presumptant state, islet DNA of G\(_{i2}\)-B\(_{i2ko}\) 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\(_{i2}\)-B\(_{i2ko}\) hypothalami. 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\(_{i2}\) protein in pancreatic islets was studied by immunoblot analysis. Using specific antibodies against the COOH-terminal region of G\(_{i2}\), we detected the G\(_{i2}\) protein in islet homogenates of WT and ctrl mice, whereas G\(_{i2}\) was strongly reduced by ~90% in G\(_{i2}\)-B\(_{i2ko}\) animals (Fig. 1, C and D), and absent in G\(_{i2}^{-/-}\) islet lysates (Fig. 1C). Remaining G\(_{i2}\) immunoreactivity in G\(_{i2}\)-B\(_{i2ko}\) likely results from the expression of G\(_{i2}\) in other endocrine cell types i.e., α-, β-, δ-, ε-, or PP-cells (see below). To further verify the cell-specific ablation of G\(_{i2}\) in G\(_{i2}\)-B\(_{i2ko}\) tissues, immunoblot analysis of hypothalamic lysates from ctrl and G\(_{i2}\)-B\(_{i2ko}\) animals were performed. G\(_{i2}\) protein was absent in hypothalamic protein extracts from G\(_{i2}^{-/-}\) mice (Fig. 1E), whereas the G\(_{i2}\) protein levels of G\(_{i2}\)-B\(_{i2ko}\) were unaffected, i.e., at control levels (Fig. 1, E and F). The combined analysis of G\(_{i2}\)-B\(_{i2ko}\) tissues by genomic PCRs 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\(_{i2}\) 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\(_{i2}\) staining was restricted in proximity to the plasma membrane. Insulin (blue) was found mainly in the cytoplasm in ctrl, G\(_{i2}\)-B\(_{i2ko}\), and G\(_{i2}^{-/-}\) islets (left). In ctrl islets, G\(_{i2}\) 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\(_{i2}\)-B\(_{i2ko}\) islets showed the specific membrane-close G\(_{i2}\) staining (middle, arrows). These G\(_{i2}\)-positive cells were all identified as insulin negative and therefore non-β-cells (middle right). In G\(_{i2}^{-/-}\), pancreata, the membrane-close G\(_{i2}\) staining was absent in all islet cells, insulin-negative and insulin-positive ones (bottom middle and bottom right). These results validated that G\(_{i2}\) and insulin could be found only in ctrl β-cells, whereas G\(_{i2}\)-B\(_{i2ko}\) β-cells lacked the G\(_{i2}\) protein. Therefore, a successful recombination in G\(_{i2}\)-B\(_{i2ko}\) islets was restricted to β-cells and demonstrated on a cellular level.

Analysis of G protein subunit levels in G\(_{i2}^{-/-}\) and G\(_{i2}\)-B\(_{i2ko}\) 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\(_{i2}\) isoforms in pancreatic islets as well as G\(_{o}\) and G\(_{q}\) has been described. All of them perform major functions in the insulin secretion machinery. To get insight into possible upregulation and compensation in G\(_{i2}\) deficiency by other G protein family members, G\(_{i2}^{-/-}\) (Fig. 2) and G\(_{i2}\)-B\(_{i2ko}\) (Fig. 3) islet homogenates were immunoblotted for putative changes in the expression of G\(_{q}\) (Fig. 3, A and B) and G\(_{q}\)-B\(_{qko}\) (Fig. 3, C). The expression levels of the G\(_{o}\), G\(_{q}\), G\(_{q}\), G\(_{q}\)-B\(_{qko}\), and G\(_{q}\)-B\(_{qko}\) isoforms remained at the control values in G\(_{i2}^{-/-}\) (Fig. 2, A–C) and G\(_{i2}\)-B\(_{i2ko}\) islets (Fig. 3, A–C). In contrast, the highly homologous isoform G\(_{q}\) was significantly upregulated in G\(_{i2}^{-/-}\) islets (Fig. 2D) (146.3 ± 43.3% of wt, P < 0.001). Importantly, in G\(_{i2}\)-B\(_{i2ko}\) islets, G\(_{q}\) 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\(_{q}\) expression levels, the receptor-dependent activation of G\(_{q}\) results in the release of significant amounts of βγ-complexes (4). Moreover, it has been shown that stabilization of GDP-bound Go by Gβγ is likely a major mechanism for the maintenance of stoichiometry between G\(_{q}\) and Gβγ (9, 18). Therefore, an important point was to investigate whether expression levels of the β-isofoms were altered in G\(_{i2}^{-/-}\) and G\(_{i2}\)-B\(_{i2ko}\) islets. Unaltered Gβ-protein levels, but simultaneously reduced G\(_{i2}\) protein levels, might result in permanently free βγ-complexes and increased βγ-mediated effects. This could mask the physiological consequences of G\(_{i2}\) deletion. At least five different Gβ subunits (Gβ\(_{1}\)–Gβ\(_{5}\))
have been described (13), with β1 and β2 being predominantly and ubiquitously expressed. In both mutant mouse lines, lower expression of Gβ1 (Gα2β2−/− 64.3 ± 9.6% of wt, P = 0.06; Gα2β2−/− 61.1 ± 19.5% of ctrl, P = 0.1) and Gβ2 (Gα2β2−/− 80.3 ± 4.3% of wt, P < 0.01; Gα2β2−/− 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 Gβ2 levels was more prominent in Gα2β2−/− islets than in Gα2β2−/− islets, most likely due to increased Gα12 protein levels (145%) in Gα2β2−/− islets. The reduced Gβ protein levels let it seem rather unlikely that effects observed in the gene-targeted mice resulted from increased Gβγ complex activity.

Metabolic evaluation of Gα2β2−/− and Gα2β2cko mice. To study the consequences of Gα12 deletion on the metabolism, we performed metabolic analysis of WT vs. Gα2β2−/− (Fig. 4) and ctrl vs. Gα2β2cko (Fig. 5) mice. Global Gα12 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 Gα12 deletion on the glucose homeostasis of these animals. Global deletion of Gα12 did not affect blood glucose levels during the glucose challenge.

Fig. 1. Gα12 expression analysis in β-cell-specific (Gα2β2cko) and globally Gα12-deficient (Gα2β2−/−) mice. A: schematic overview of the Gna12 gene and PCR strategy for recombination analyses. B: genomic DNA analysis of heart, islets, and hypothalamus isolated from control (ctrl) and Gα2β2cko animals. Recombination is restricted to Gα2β2cko islets. C: representative immunoblots of islet homogenates isolated from ctrl, Gα2β2cko, wild-type (wt), and Gα12−/− mice. Gα12 protein is almost absent in Gα2β2cko islets and completely absent in Gα12−/− islets. The weak top band (*) might result from Gα11 expression in islets. Equal loading was confirmed by β-actin detection. D: statistical analysis of Gα12 expression levels in ctrl and Gα2β2cko islets. Islets from 4 animals were analyzed in ≥3 independent experiments. E: representative immunoblot analysis of hypothalamic homogenates isolated from ctrl and Gα2β2cko mice. As a negative control, hypothalamic homogenates from Gα12−/− mice were loaded. The top band (*) might result from Gα11 expression in the brain. Equal loading was confirmed by β-actin detection. F: statistical analysis of Gα12 expression levels in hypothalami of ctrl and Gα11βκαα mice. Hypothalamic tissue from 4 animals was analyzed in ≥3 independent experiments. G: representative immunostainings of pancreatic cryosections stained against Gα12 (yellow) and insulin (blue). Gα12 shows proximity to the plasma membrane, whereas insulin is mainly found in the cytoplasm. In ctrl islets, Gα12 staining is detectable in both insulin-positive β-cells and insulin-negative cells. Gα12 protein is absent in Gα2β2cko β-cells but can be observed in insulin-negative cells. Gα12 staining is completely absent in all Gα2β2−/− islet cells. Diffuse yellow staining is background staining. Scale bars, 20 μm.
Fig. 2. Statistical analysis of different Go- and Gβ-subunit expression patterns in Go12−/− islets. Go12, Go12 (A), Gαs (common), Gαs (B), and Gβ4 (C) expression levels are not affected by Go12 deletion in Go12−/− islets. D: Go12 protein upregulation is detectable in Go12−/− islets. To verify Go13 antibody specificity, Go13−/− islets were loaded. E: Gβ4 and Gβ5 protein levels are downregulated in Go12−/− islets. Gβ2 downregulation gains statistical significance. Inserts: representative immunoblots of islet homogenates from wt and Go12−/−. Equal loading was confirmed by β-actin or α-tubulin detection.

(Fig. 4C). Interestingly, significantly lower insulin levels were measured in Go12−/− mice at 15, 30, and 60 min after glucose injection compared with WT mice (Fig. 4D). In addition, Go12−/− animals displayed an improvement in insulin tolerance (Fig. 4E). To assess whether β-cell-specific deletion of Go12 influences the body weight of Go12−/− animals, we recorded body weight and fasted blood glucose levels over time in Go12−/− and ctrl animals (Fig. 5, A and B). Continuous measurements did not reveal any differences between these two groups. Again, we monitored blood glucose levels following an ip injection of glucose after an overnight fasting period in the Go12−/− and ctrl mice (Fig. 5C). β-Cell-specific Go12 deletion caused significantly increased blood glucose levels in the Go12−/− mice 30, 60, and 120 min after the glucose challenge, pointing to an impaired glucose tolerance. The parallel analysis of plasma insulin levels during the glucose tolerance test revealed significantly lower plasma insulin levels at 15, 30, and 60 min in Go12−/− mice vs. the littermate controls (Fig. 5D). Consistent with the reduced plasma insulin, plasma C-peptide levels were significantly decreased at 15 and 30 min after the glucose bolus in Go12−/− mice (Fig. 5E). Insulin sensitivity, tested by the injection of insulin to 4-h-fasted Go12−/− and ctrl mice, revealed no differences between the two groups (Fig. 5F). These findings indicate that Go12 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, Go12 deletion restricted to β-cells lowered plasma insulin levels. Collectively, these results indicate that deletion of Go12 in β-cells impairs glucose tolerance by decreasing insulin secretion, suggesting that Go12 is a positive regulator of insulin secretion.

Islet function in vitro. In Go12−/− mice, Go12 deletion is restricted to β-cells, Go13 is not upregulated in this conditional gene-targeted mouse line, and body weight is not affected by β-cell Go12 deletion. Thus, these mice are an appropriate model for studying the molecular mechanism of Go12 signaling in isolated islets independently of systemic effects. No abnormalities were detected in morphometric analysis of Go12−/− islets. Islet area, number of nuclei and islet size (Fig. 6, A–E), pancreas and islet insulin content, and whole islet DNA content were indistinguishable between ctrl and Go12−/− islets (Fig. 6, F–H). We performed insulin secretion studies on islets isolated from Go12−/− and ctrl mice. Insulin release was studied either

AJP-Endocrinol Metab • doi:10.1152/ajpendo.00337.2014 • www.ajpendo.org
under low (3 mM) or high (16 mM) glucose concentrations. All G\(_{\alpha_1}\)\(_{\text{b}}\)\(_{\text{c}}\)\(_{\text{k}}\) and ctrl islets 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 G\(_{\alpha_1}\) in G\(_{\alpha_1}\)\(_{\text{b}}\)\(_{\text{k}}\) islets (Fig. 7A). Since a large body of PTx-based studies suggested that G\(_{\alpha_1}\)\(_{\text{b}}\) 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 G\(_{\alpha_1}\) in G\(_{\alpha_1}\)\(_{\text{b}}\)\(_{\text{k}}\) islets (Figs. 7, B and C). This argues against an inhibitory role of G\(_{\alpha_1}\) in the regulation of insulin secretion from \(\beta\)-cells. In contrast, significantly reduced in vivo plasma insulin levels in G\(_{\alpha_1}\)\(_{\text{b}}\)\(_{\text{k}}\) mice suggest that G\(_{\alpha_1}\) 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 islet GPCRs (37). To identify the stimulus, we analyzed the effect of the free fatty acid palmitate on insulin secretion in G\(_{\alpha_1}\)\(_{\text{b}}\)\(_{\text{k}}\) islets. Simultaneous stimulation of insulin with high glucose and palmitate (200 \(\mu\)M) resulted in increased insulin secretion in ctrl and G\(_{\alpha_1}\)\(_{\text{b}}\)\(_{\text{k}}\) 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 G\(_{\alpha_1}\)\(_{\text{b}}\)\(_{\text{k}}\) islets (Fig. 8B). However, application of L-arginine and L-ornithine surprisingly produced significantly differing insulin secretion responses in ctrl and G\(_{\alpha_1}\)\(_{\text{b}}\)\(_{\text{k}}\) islets. Costimulation of L-arginine (10 mM) together with glucose (16 mM) showed decreased insulin release from G\(_{\alpha_1}\)\(_{\text{b}}\)\(_{\text{k}}\) 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 G\(_{\alpha_1}\)\(_{\text{b}}\)\(_{\text{k}}\) 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 mg/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 Goα_{2}^{i/kko} 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 Goα_{2} isoform is not an inhibitor but a stimulator of insulin secretion. 

\[ \text{[Ca}^{2+}]_{i} \text{ measurements in islets.} \] 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 \([\text{Ca}^{2+}]_{i}\). This can be determined in single islet cells and is therefore a cell-based correlate for β-cell insulin secretion. Measurements of intracellular calcium changes link the Goα_{i}-dependent signaling to insulin secretion at a cellular level. Changes in \([\text{Ca}^{2+}]_{i}\), 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 Goα_{2}^{i/kko} single islet cells showed a similar increase in \([\text{Ca}^{2+}]_{i}\), in response to the application of 16 mM glucose (Fig. 9A). The Ca^{2+} response was significantly reduced in Goα_{2}^{i/kko} 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-arginine did not induce a calcium response either in Goα_{2}^{i/kko} or in ctrl single islet cells (Fig. 9C). In contrast, the L-arginine-induced response was reduced by 25% (Fig. 9E, left). The reduced response to L-arginine of Goα_{2}^{i/kko} cells was even more evident when β-cells were coexposed to high glucose concentrations and L-arginine (Fig. 9D). The L-arginine-induced increase in \([\text{Ca}^{2+}]_{i}\) 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 cotreatment resulted in a robust increase in \([\text{Ca}^{2+}]_{i}\) in control islets, whereas the response of Goα_{2}^{i/kko} islet cells was signif-
AMINO ACID-STIMULATED INSULIN SECRETION DEPENDS ON G\(\alpha_{i2}\)

E807

Fig. 5. Metabolic phenotyping of G\(\alpha_{i2}\)-null mice. A: body weight determined in ctrl and G\(\alpha_{i2}\)-null mice over time. Body weight gain was similar in ctrl and G\(\alpha_{i2}\)-null mice (n = 8 animals per genotype). B: fasted blood glucose levels monitored in ctrl (\(\square\)) and G\(\alpha_{i2}\)-null (\(\triangledown\)) mice over time. C: blood glucose levels of 8- to 10-wk-old ctrl and G\(\alpha_{i2}\)-null mice following ip application of glucose after overnight fasting (n = 16 animals per group). G\(\alpha_{i2}\)-null mice have impaired glucose tolerance. Plasma insulin (D) and plasma C-peptide (E) levels during ip glucose tolerance testing were significantly reduced in G\(\alpha_{i2}\)-null mice (n = 5–8 animals per group). F: insulin secretion test in 4-h-fasted ctrl and G\(\alpha_{i2}\)-null mice (n = 8 animals per group). *\(P < 0.05, **P < 0.01, ***P < 0.001.

Importantly, the selective and specific stimulatory effects of \(L\)-arginine on insulin secretion in vitro and in vivo. Although PTx-based studies in mice and isolated islets assumed an inhibitory role for all G\(\alpha_i\) and G\(\alpha_o\) proteins, our data surprisingly indicate that G\(\alpha_{i2}\) 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 G\(\alpha_{i2}\) 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

significantly reduced by 44% (Fig. 9E, right). The Ca\(^{2+}\) responses evoked by high K\(^+\) were similar in ctrl and G\(\alpha_{i2}\)-null \(\beta\)-cells (Fig. 9, D and F). These analyses identify \(\beta\)-cell G\(\alpha_{i2}\) as necessary for the stimulation of insulin secretion by \(L\)-arginine in a calcium-dependent manner but independent of a depolarization that may be caused by the positive charge of the amino acid.

DISCUSSION

The present study analyzed the physiological role of G\(\alpha_{i2}\) in insulin-secreting \(\beta\)-cells by using global and \(\beta\)-cell-specific G\(\alpha_{i2}\) mutants. Significantly lower plasma insulin levels were observed upon glucose challenge in both the G\(\alpha_{i2}\)-null and the G\(\alpha_{i2}\)-null 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 G\(\alpha_{i2}\)-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 G\(\alpha_{i2}\) deficiency. This study provides causal evidence for the involvement of G\(\alpha_{i2}\) 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 G\(\alpha_i\) and G\(\alpha_o\) proteins, our data surprisingly indicate that G\(\alpha_{i2}\) proteins rather stimulate than inhibit the secretion of insulin by regulating \(L\)-arginine- and \(L\)-ornithine-induced insulin secretion in vitro and in vivo.
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<sup>Δlck</sup> 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 Gα<sub>o</sub> mice (42). Those authors suggest that Gα<sub>o</sub> is a strong inhibitor of insulin secretion, since its ablation produced a strong stimulatory effect on insulin release. Gα<sub>o</sub> 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<sup>Δlck</sup> islets. Of course, we are aware of the fact that these findings conflict with the expected functions for Gα12 based on PTx experiments, a pan inhibitor of Gα<sub>o</sub> and Gα<sub>12</sub> proteins and therefore a strong stimulator of insulin secretion. However, clonidine, an α2-adrenoreceptor agonist, completely inhibited...
insulin release in control and Go\(_{12}\)\(^{-}\)deficient islets. Thus, our data set provides additional evidence that Go\(_{12}\) is not involved in the \(\alpha_2\)-adrenoreceptor-mediated signaling. The inhibition of the inhibitory Go\(_{12}\)—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 Go\(_{12}\)-deficient animals (42). This suggests that, despite the fact that Go\(_1\) proteins being expressed in islet cells and ADP-riboseylated by PTX, Go\(_1\) is the major target for PTX stimulating insulin secretion, thereby making an inhibitory role of Go\(_{12}\) very unlikely (42). A single study shows an inhibitory role for Go\(_{12}\) in ghrelin-dependent inhibition of insulin secretion based on an antisense oligonucleotide approach (5). Our data demonstrate that deletion of Go\(_{12}\) resulted in normal glucose-induced insulin secretion per se, but stimulatory effects of amino acids like L-arginine and L-ornithine on insulin secretion induced insulin secretion were diminished in Go\(_{12}\)\(^{-}\)deficient islets. Moreover, Go\(_{12}\)\(^{-}\)deficient 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 Go\(_{12}\) 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 Go\(_{12}\)-deficient animals, which was seen in littermate controls.

Our detailed analysis of G protein levels in the Go\(_{12}\) gene-targeted mouse lines corroborates significant upregulation of Go\(_{13}\) (about 45%) in the global Go\(_{12}\)-deficient mice, which has also been described for other tissues and organs (6, 17, 39, 40). In contrast to the global Go\(_{12}\)-deficient islets, the Go\(_{13}\) protein levels were unchanged in Go\(_{12}\)\(^{-}\)deficient islets. Since plasma insulin levels are reduced in both gene-targeted mouse lines, Go\(_{13}\) is obviously not able to compensate for the lack of Go\(_{12}\). This confirms previous reports on specific and distinct functions of Go\(_{12}\) and Go\(_{13}\) (8, 10, 17). Reduced Gβ expression levels parallel deletion of Go\(_{12}\). Heterotrimeric G proteins are stoichiometrically composed of Gα and Gβγ, which dissociate selectively during the activation cycle. The stabilization of Gα and Gβγ in 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 Go\(_{12}\)-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>Go(_{12})</th>
<th>Go(_{12})(^{-})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Secreted insulin</td>
<td>Secreted insulin</td>
</tr>
<tr>
<td></td>
<td>%Insulin content</td>
<td>%Insulin content</td>
</tr>
<tr>
<td>3 mM glucose</td>
<td>0.189 ± 0.02</td>
<td>0.174 ± 0.02</td>
</tr>
<tr>
<td>3 mM glucose</td>
<td>5</td>
<td>27</td>
</tr>
<tr>
<td>1 μM clonidine</td>
<td>0.153 ± 0.05</td>
<td>0.164 ± 0.01</td>
</tr>
<tr>
<td>100 nM SST</td>
<td>0.176 ± 0.05</td>
<td>0.141 ± 0.04</td>
</tr>
<tr>
<td>200 mM palmitate</td>
<td>0.324 ± 0.04</td>
<td>0.470 ± 0.02</td>
</tr>
<tr>
<td>100 μM L-leucine</td>
<td>0.292 ± 0.05</td>
<td>0.329 ± 0.06</td>
</tr>
<tr>
<td>10 mM L-arginine</td>
<td>0.075 ± 0.02</td>
<td>0.143 ± 0.03</td>
</tr>
<tr>
<td>10 mM L-ornithine</td>
<td>0.150 ± 0.02</td>
<td>0.039 ± 0.01</td>
</tr>
</tbody>
</table>

n.s., not significant; n, set of triplicates.

---

Fig. 7. Insulin secretion experiments in ctrl and Go\(_{12}\)\(^{-}\) islets. A: glucose-induced insulin secretion measured at 3 and 16 mM glucose and 30 mM KCl (3 mM glucose: ctrl \(n = 60\); Go\(_{12}\)\(^{-}\) \(n = 59\); n, number of incubations). Inhibitory effect of clonidine (B) and SST (C) on glucose-induced insulin secretion (1 μM clonidine: ctrl \(n = 15\); Go\(_{12}\)\(^{-}\) \(n = 14\)) was not affected by deletion of Go\(_{12}\) in β-cells. All conditions were performed in islet preparations derived from ≥3 animals per genotype.
mouse lines, with a more pronounced reduction of Gβ levels in Gα2β2cko islets (see Fig. 3E) than in Gα2β2−/− islets (see Fig. 2E). It is reasonable to assume that the differences in the extent of reduction between Gα2β2−/− and Gα2β2cko islets result from the selective upregulation of Gα3 protein in Gα2β2−/− 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 Go 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 Gβ2 is the predominant isoform in islets, which has been reported only for human placenta (23). Signaling by the Go family of “inhibitory” proteins is complex. Go proteins can activate or inhibit many other effectors through either the GTP-bound Go or the released Gβγ dimer upon activation. Therefore, it is important to note that both the abolished signaling of Gα2β2 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 Gα2β2cko 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 Gα2β2−/− mutants. In line with this, Gα2β2−/− deficient mice showed improved insulin tolerance, arguing for the improved peripheral insulin sensitivity. The lean phenotype was not observed in Gα2β2−/− mutants. Control and mutant animals showed similar weight gain. However, Gα2β2cko animals showed impaired glucose tolerance caused by impairment of the β-cells’ ability to secrete the required amount of insulin. The differences in
blood glucose levels between global and β-cell-specific Gα12 mice imply that the metabolic functions of Gα12 in the body are broader than previously thought. Gα12 signaling might play a role for glucose homeostasis in liver, white adipose tissue, or skeletal muscle as well, but Gα12 signaling is also important for efficient pancreatic β-cell function. Thus, we propose that the diminished plasma insulin levels indeed result from islet Gα12 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 Gα12-/-cells strengthens the finding that Gα12 stimulates insulin secretion in a calcium-dependent manner. However, it remains elusive whether regulation of intracellular calcium release and/or extracellular calcium influx is disturbed by the Gα12 deletion.

In conclusion, the experiments presented identify Gα12 as an endogenous positive modulator of the L-arginine- and L-ornithine-induced stimulation of insulin secretion, which may be an important feedback mechanism for fine-tuning amino acid plasma levels and insulin secretion. Interestingly, the PTx-sensitive Gα12 isoform was identified as a stimulator of insulin secretion. This strengthens the hypothesis that Gαδ and Gαo proteins have distinct, nonredundant, and importantly opposite roles in pancreatic β-cells: 1) Gαδ isoforms function as inhibitors of insulin secretion and are the main isoform being responsible for the dysregulated insulin release upon PTx treatment; and 2) Gα12 isoforms stimulate insulin secretion upon L-arginine and L-ornithine treatment.

ACKNOWLEDGMENTS

We kindly thank Pedro Herrera, University of Geneva, for providing us with the Rip-Cre mouse line. We express our appreciation to Hans-Ulrich Häring and Susanne Ulrich for critical discussion of results and manuscript. We thank Lennart Fiedler, Anna-Floriane Hennig, and Anna-Elisa Glaser for genotyping the mice. We acknowledge the support of U. Schmidt, and B. Schreiner for assistance at the BioPlex 200 System. We thank Bianca Walter for performing morphological analysis.

GRANTS

This work was supported by the Deutsche Forschungsgemeinschaft (DFG) with grants to B. Nürnberg and V. Leiss, and GRK 1302 to B. Nürnberg, by the Intramural Program of the National Institutes of Health to L. Birnbaumer (Project Z01 ES-101684), and by the German Ministry of Education and Research (BMBF: DZD, 01GI0922) to A. Schürmann.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

REFERENCES


8. E812 AMINO ACID-STIMULATED INSULIN SECRETION DEPENDS ON Gαi2


