Online Appendix - Methods

Quantitative RT-PCR

Human pancreatic islets were hand-picked (200-400 per preparation) and total RNA was purified using RNeasy Mini kit (Qiagen) followed by DNase digestion (RQ1 DNase, Promega). Spectrophotometric $A_{260/280}$ ratios spanned 1.99-2.09 (Nanodrop ND-1000, Nanodrop Technologies). 20 µl reverse transcription reactions containing (final concentrations shown) 0.5 mM dNTP, 2 µM oligo(dT), 2 µM random hexamers (all Applied Biosystems) and 1–4 µg of total RNA were incubated at 65°C for 5 min, followed by addition of 1X First-Strand buffer, 5 mM DTT, 20 U RNaseOUT RNase inhibitor and 200 U SuperScript III (all Invitrogen). Samples were incubated at 25 °C for 5 min and then at 50 °C for 1 hr. Quantitative real-time PCR was performed using an Applied Biosystems 7900HT in 10 µl reactions containing 4 µl diluted (1:50) cDNA samples, 1X SYBR Green JumpStart Taq ReadyMix, 2X Reference dye for qPCR (both Sigma) and 0.2 µM forward and reverse oligonucleotide primers (Invitrogen). Intron-spanning primers were designed with Primer3Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) using sequences from Ensembl release 45 (June 2007) (Table S1). Specificity of the assays was confirmed with reverse ePCR (NCBI). Constant reaction efficiencies and fluorescent signal to amplicon copy number ratios for all assays are assumed. All data were normalized to the expression level of the reference gene ribosomal protein S29 (RS29).

Table S1. PCR primer sequences

Gene ID	Forward primer	Reverse primer	Amplicon length (bp)
GABRA1	CCTCCTTCTGAGCACACTGAC	CTGTCCAAAATCCTGGTGAAG	99
GABRA2	TCCTGCTTTTTGTTTTCTTGG	TTTAGCCTCATCTTCTTGGATGT	74
GABRA3	AGGCAAGAAGGTGCCAGAG	CCAGGTTGATGGGATAGGTG	113
GABRA4	CCAGTGCAGAGAGAGAAGCA	AATGGTTTCCCACCTCAGTTC	133
GABRA5	GATCCTCCAGCCCAGAGAC	CAAAGCCAGCAAGCTACTCC	75
GABRA6	CTGAACCTTTGGAAGCTGAGA	TTATTGGCCTCGGAAGATGA	109
GABRB1	CAAGACCAGAGTGCCAATGA	CAGGGTGCTGAGGAGAATGT	87
GABRB2	CCAGTGCCAACAATGAGAAG	AAGTCCCATCACAGCCTCA	122
GABRB3	AAGACAGCCAAGGCAAAGAA	CTTCCAGCGATGTCAACAGA	88
GABRG1	CCTTTTCTTCTGCGGAGTCA	AGACCCAGGTTTTGTTCACC	133
GABRG2	GTGGATTCTGCTCCTGCTGT	TCAAGACCCATGTTTTGTTAGAAG	98
GABRG3	TCCTGAGCGAATAAGCCTACA	CAGTCTTTGCCATCCAGACA	150
GABRD	ACGACGTGACGGTGGAG	ACGATGTCCTCCGATGAGTAA	175
GABRE	ACAGGAGTGAGCAACAAAACTG	TGAAAGGCAACATAGCCAAA	107
GABRP	GCCCTCAGAATCACGACAA	GCACAGAGTCGTTCCCTCTC	148
GABRQ	GTGCATGATGTGACTGTGGAG	ATGCAGATCCAGGGAACAAG	108
RS29	GCCAGGGTTCTCGCTCTT	CGCGTACTGACGGAAACAC	98

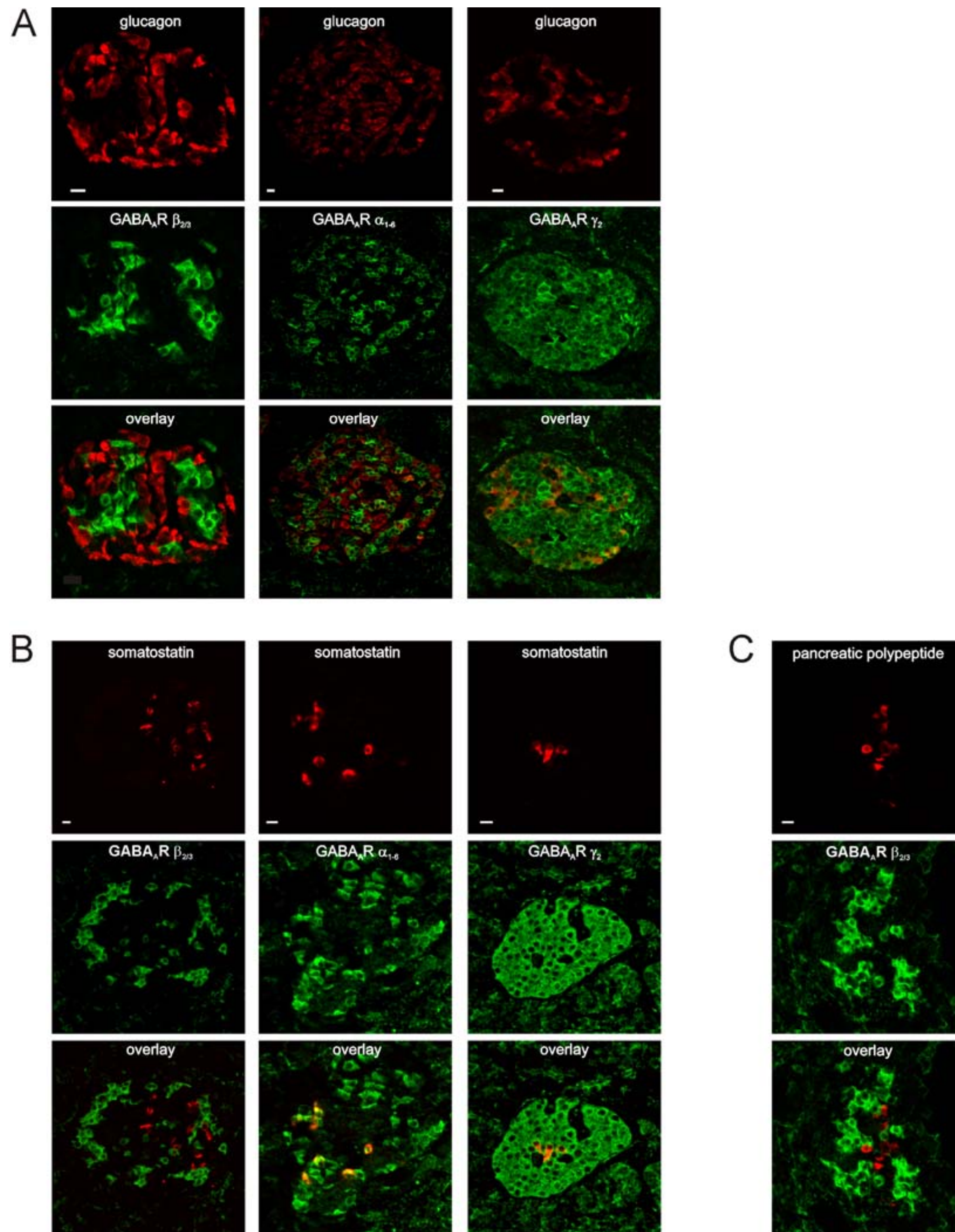
©2010 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/cgi/content/full/db09-0797/DC1.

Identification of cell types after patch-clamping by immunocytochemistry

After the experiment, the location of the patch-clamped cell was marked in the Petri-dish. The cells were then fixed with 4% paraformaldehyde for 10 min. After washing with PBS, the cells were permeabilized in 5% goat serum containing 0.1% Triton X-100 for 15 min. This was followed by incubation with guinea-pig anti-insulin (Abcam, Cambridge, UK; dilution 1:200), mouse anti-glucagon (Sigma-Aldrich; dilution 1:2000) and rabbit anti-somatostatin antibodies (dilution 1:100) in 5% goat serum for 2 h at room temperature. The cells were washed with PBS and incubated with fluorophore-conjugated goat secondary antibodies (anti guinea-pig Alexa-633, anti-mouse Alexa-488 and anti-rabbit Alexa-568, all from Invitrogen, dilution 1:500 in 5% goat serum) for 1 h at room temperature. When biocytin-containing intracellular solution was used, one of the secondary antibodies was omitted, and the cells were additionally incubated for 15 min with Alexa-488 or Alexa-546 conjugated streptavidin (1:500 in PBS, Invitrogen). Fluorescence was visualized in an upright confocal microscope (Zeiss Axioskop 2) equipped with a Zeiss LSM 510 Meta scanning module.

©2010 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/cgi/content/full/db09-0797/DC1.

Online Appendix – Figure 1

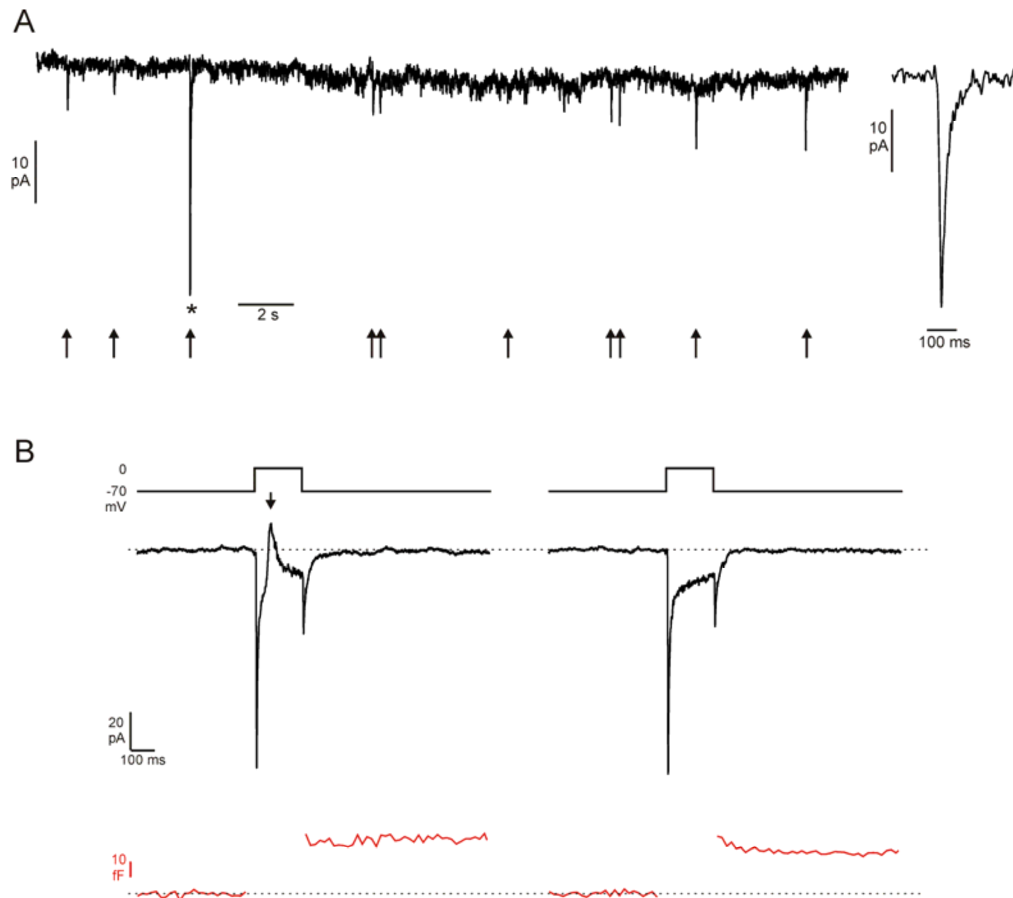


Expression of GABA$_A$R subunits in non-β-cells in human pancreatic islets

A) Co-labelling of human pancreatic tissue sections with anti-glucagon (top, red) and anti-GABA$_A$R $\beta_{2/3}$ (left), anti-GABA$_A$R α_{1-6} (middle) or anti-GABA$_A$R γ_2 (right). The bottom row shows the overlay of both signals. **B)** Co-labelling of human pancreatic tissue sections with anti-somatostatin (top, red) and GABA$_A$R-subunit specific antibodies as indicated, with the bottom row showing merged signals. **C)** Co-staining of a human pancreatic tissue section anti-pancreatic polypeptide and anti-GABA$_A$R $\beta_{2/3}$. Scale bars: 10 µm.

©2010 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/cgi/content/full/db09-0797/DC1.

Online Appendix – Figure 2



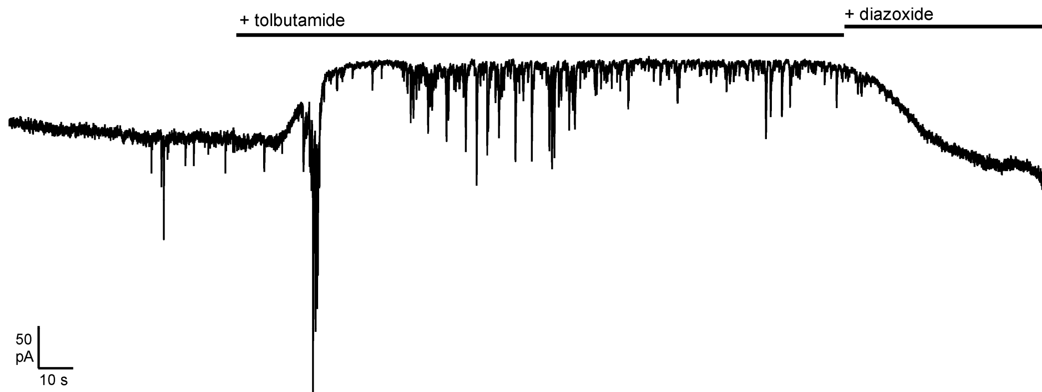
Vesicular GABA release from human δ-cells

A) A δ-cell that had been infected with adenoviral vectors to over-express $GABA_AR$ α_1/β_1 was infused with solution containing 2 μM free Ca^{2+} via the patch pipette and clamped at -70 mV. Transient inward currents corresponding to vesicular GABA release are indicated by arrows. The event marked by the asterisk is shown on an expanded time base on the right.

B) A non-infected δ-cellwas stimulated by 200 ms voltage-clamp depolarizations from -70 to 0 mV, using an intracellular solution containing 50 μM EGTA. A GABArelease induced transient outward current is indicated by the arrow. For comparison, a trace without GABA-induced current is shown on the right. The red traces represent the capacitance responses that were measured simultaneously. The observed increase of ~30 fF corresponds to the release of ~30 secretory granules (assuming that each somatostatin granule contributes 1 fF of capacitance; Gopel et al. 2004, J. Physiol. **556**, 711-726), one of which apparently contained GABA.

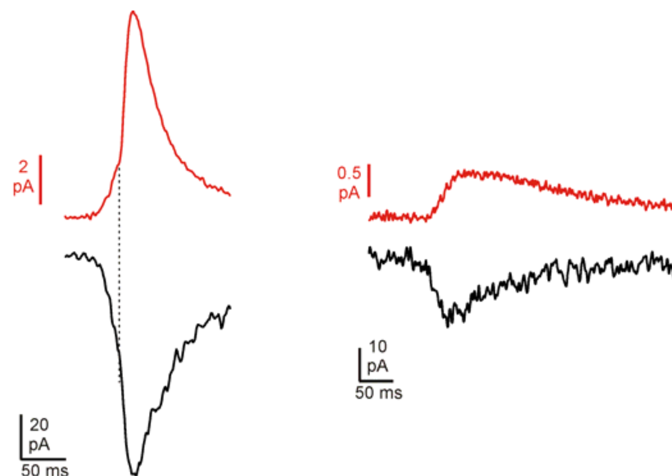
©2010 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/cgi/content/full/db09-0797/DC1.

Online Appendix – Figure 3



GABA release from β-cells is stimulated by tolbutamide and inhibited by diazoxide
A cell (within a cell cluster) over-expressing $GABA_AR$ α_1/β_1 subunits was held at -70 mV and infused with intracellular solution containing 10 mM EGTA. The bath solution contained 10 mM glucose. Addition of tolbutamide (100 µM) increased the frequency of GABA-induced TICs, while addition of diazoxide (100 µM) led to a complete inhibition of GABA release. Tolbutamide also inhibited the K_{ATP} conductance remaining in the patch-clamped cell despite the presence of 3 mM ATP in the intracellular solution, thus reducing the background current. This effect was reversed by diazoxide.

Online Appendix – Figure 4

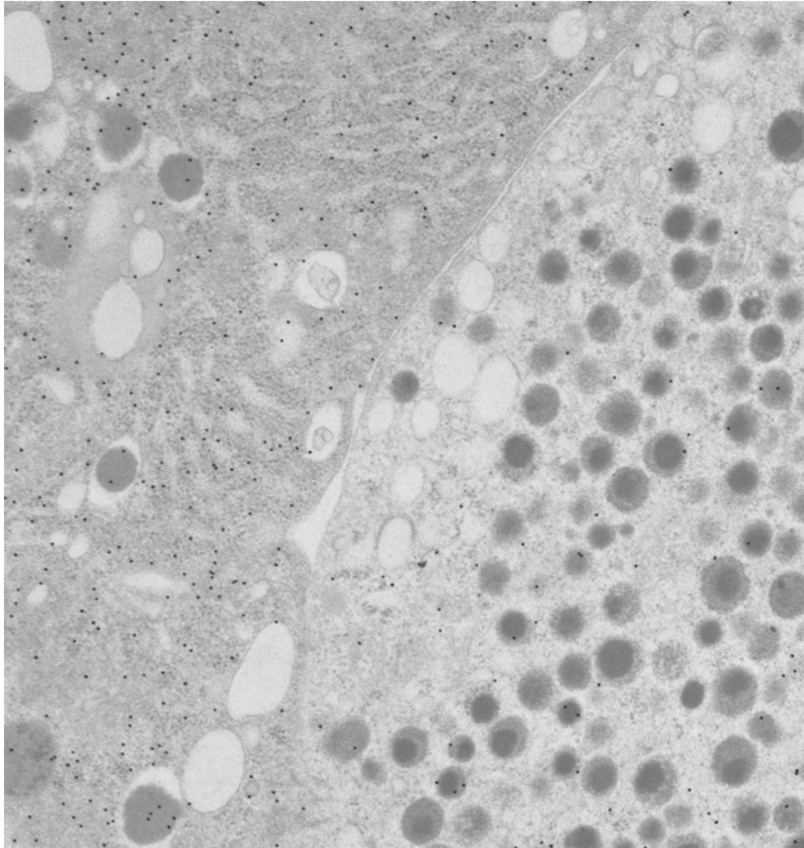


GABA release through fusion pores of LDCVs
A β-cell over-expressing $GABA_AR$ α_1/β_1 was infused with intracellular solution containing 2 µM free Ca^{2+} through the patch pipette and clamped at -70 mV. The black traces represent the membrane current and display transient inward currents corresponding to vesicular GABA-release. The red traces represent amperometric

©2010 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/cgi/content/full/db09-0797/DC1.

currents that were simultaneously measured in the same cell using a carbon fibre electrode, and that display events caused by serotonin release from LDCVs. The event on the left shows a foot signal preceding the spike in both traces (the transition from foot to spike signal is indicated by the vertical dashed line). The event to the right displays slow kinetics in both traces, compatible with a "stand-alone foot" that may correspond to kiss-and-run exocytosis. The traces suggest that GABA, like serotonin, can be released through the fusion pore of LDCVs in human β-cells.

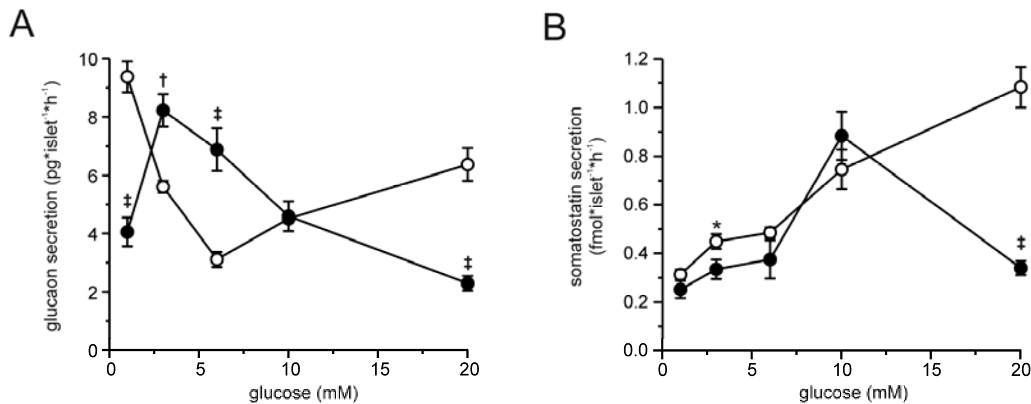
Online Appendix – Figure 5



Localization of GABA in human islets
Immunogold electron micrograph showing a human β-cell (left) and a α-cell (right) labelled with anti-GABA. The labelling intensity (excluding nuclei) was 29.4±2.5 gold particles/μm^2 in β-cells (n=23 cells), 16.4±2.7 particles/μm^2 in α-cells (n=13), 20.1±6.2 particles/μm^2 in δ-cells (n=4) and 11.6 particles/μm^2 in PP-cells (n=2). The average value for non-β-cells was 16.7±2.2 ($p<0.001$ vs. β-cells). For comparison, rat β-cells labelled with the same technique contained 12.8±2.1 gold particles/μm^2, whereas the respective value for non-β-cells was 2.8±0.6 (n=8). The relatively high GABA-content of human non-β-cells is in agreement with a report that (unlike in rat islets) expression of GAD65 is not confined to β-cells in human islets (Petersen JS et al. 1993, Diabetes 42:484).

©2010 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/cgi/content/full/db09-0797/DC1.

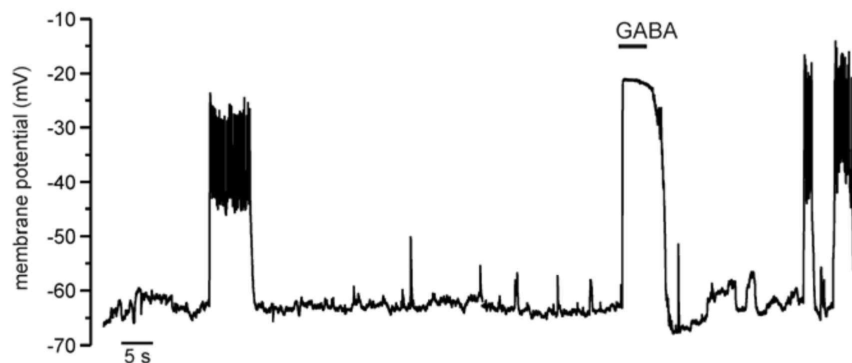
Online Appendix – Figure 6



Effect of $GABA_A R$ blockade on glucagon and somatostatin secretion from isolated human islets.

Secretion of glucagon (**A**) and somatostatin (**B**) was measured at 1, 3, 6, 10 or 20 mM extracellular glucose in the absence (open circles) or presence (closed circles) of SR-95531 (10 µM) as indicated. (n=7-12 from 3-4 donors; *$p<0.05$, †$p<0.01$, ‡$p<0.001$ for the effects of SR-95531). Apart from somatostatin secretion at 6 vs. 3 mM glucose, hormone release at all glucose concentrations was significantly different from the previous lower glucose concentration (not indicated). The molecular weight of somatostatin 14 (the isoform secreted from the pancreas) is ~1.600 kDa. The results at 1, 6 and 20 mM glucose were repeated with islets from 7 different donors with the same results.

Online Appendix – Figure 7



Effect of GABA on the membrane potential of δ-cells.

Membrane potential was recorded at 6 mM extracellular glucose in the perforated-patch configuration, using the Cl^--impermeable antibiotic gramicidin as the perforating agent. GABA (100 µM) was applied as indicated by the bar to a δ-cell displaying bursting electrical activity.

©2010 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/cgi/content/full/db09-0797/DC1.