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$GABA_A$ Receptor-Mediated Currents and Hormone mRNAs in Cells Expressing More Than One Hormone Transcript in Intact Human Pancreatic Islets

Sergiy V. Korol *, Zhe Jin and Bryndis Birnir

Department of Medical Cell Biology, Uppsala University, BMC, Box 593, 75124 Uppsala, Sweden; Zhe.Jin@mcb.uu.se (Z.J.); Bryndis.Birnir@mcb.uu.se (B.B.)

* Correspondence: Sergiy.Korol@mcb.uu.se; Tel.: +4618-471-4034

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Abstract: In pancreatic islets, the major cell-types are α, β and δ cells. The γ-aminobutyric acid (GABA) signalling system is expressed in human pancreatic islets. In single hormone transcript-expressing cells, we have previously characterized the functional properties of islet $GABA_A$ receptors ($iGABA_ARs$). Here, we extended these studies to islet cells expressing mRNAs for more than one hormone and sought for correlation between $iGABA_AR$ activity level and relative mRNA expression ratio. The single-cell RT-PCR in combination with the patch-clamp current recordings was used to examine functional properties of $iGABA_ARs$ in the multiple hormone mRNA-expressing cells. We detected cells expressing double (α/β, α/δ, β/δ cell-types) and triple (α/β/δ cell-type) hormone transcripts. The most common mixed-identity cell-type was the α/β group where the cells could be grouped into β- and α-like subgroups. The β-like cells had low *GCG*/*INS* expression ratio (<0.6) and significantly higher frequency of $iGABA_AR$ single-channel openings than the α-like cells where the *GCG*/*INS* expression ratio was high (>1.2). The hormone expression levels and $iGABA_AR$ single-channel characteristics varied in the α/β/δ cell-type. Clearly, multiple hormone transcripts can be expressed in islet cells whereas $iGABA_AR$ single-channel functional properties appear to be α or β cell specific.

Keywords: α-like cell; β cell; GABA; glucagon; insulin; mixed-identity cell

1. Introduction

The three major cell types of the endocrine pancreas are α, β and δ cells [1], producing glucagon (GCG), insulin (INS) and somatostatin (SST), respectively. When the physiological or pathological aspects of pancreatic islets are studied, the function of α or β cells is traditionally in the focus. However, emerging evidence indicates there are subgroups of pancreatic islet cells that previously were overlooked [2,3]. Among these are groups of cells expressing more than one hormone transcript [4–7]. They may express hormone transcripts in different combinations such as *GCG/INS*, *INS/SST*, *GCG/SST* or *GCG/INS/SST* and have different expression levels in individual cells being thus α/β, β/δ, α/δ or α/β/δ cells, respectively. Such cells are here termed "mixed-identity cells". These cells may potentially represent different developmental stages of the primary cell types [1,8] but also may appear as a consequence of exposure to different conditions, e.g., pregnancy, development of obesity or diabetes [4–7]. Altering the cell identity has been proposed to be a protecting mechanism to "camouflage" the pancreatic (β) cells from the ongoing stress induced by, e.g., type 2 diabetes [7,9].

Various voltage-gated ion channels and their effects on hormone release have been well characterized in human pancreatic α [10], β [11] and δ [12] cells. In addition to these channels, elements of the different neurotransmitter signalling machineries are found within pancreatic islets, and one of them is the GABA signalling system. Components of this system and its effects have been

detected in rodent [13,14] and also, in human [15–19] pancreatic islet cells. The GABAergic system has been shown to modulate exocytosis [17], insulin and glucagon secretion [15,16] and regulate β cell replication [18,20]. In addition, the $GABA_A$ receptors in β cells in intact human pancreatic islets and their functional properties have recently been characterized in detail [17]. Here we examined the prominence of the single and multiple hormone transcript-expressing cells within intact human pancreatic islets from non-diabetic and type 2 diabetic donors, examined patterns of activity of $iGABA_A$Rs in the mixed-identity cells and correlated the channel characteristics with the hormones' mRNA ratios. Together, the results identify the $iGABA_A$R single-channel currents as a functional marker of a subtype of the mixed-identity cells.

2. Results

2.1. Cell-Types Identified by Hormone mRNA Expression in Intact Pancreatic Islets from Non-Diabetic and Type 2 Diabetic Donors

GABA-activated single-channel currents were detected in 383 cells in intact islets from 109 donors. The cell-type was determined by single-cell RT-PCR analysis of the levels of islet insulin (*INS*), glucagon (*GCG*) and somatostatin (*SST*) transcripts for every individual cell recorded from. Hormone transcripts were detected in 174 cells from 45 non-diabetic and 8 type 2 diabetic donors (HbA1c = 6.5 ± 0.16, mean ± SEM (48 mmol/mol)). Table 1 shows the distribution of the cell-types identified. Characteristics of GABA-activated currents in the α, β and δ single-hormone cell-types have been described recently [17]. Here, we analysed the other subset of samples representing multiple hormone transcript-expressing cells. For islets from non-diabetic and type 2 diabetic donors, single-hormone transcript was detected in 55% and 48% of the cells, respectively, with 44% (non-diabetic) and 32% (type 2 diabetic donors) of the cells being insulin-positive β cells (Figure 1A). The remaining cells, 45% from non-diabetic and 52% from type 2 diabetic donors, were positive for more than one hormone transcript. The frequency of the specific subtypes of mixed-identity cells i.e., α/β, β/δ, α/δ, α/β/δ, varied somewhat between the non-diabetic and type 2 diabetic donor islets, with the most notable difference being a decrease in β/δ and an increase in mixed-identity cell subtypes expressing the *GCG* in type 2 diabetic donors (Figure 1A; Table 1). As the data from type 2 diabetic donors were limited and overlapped in values of the analysed parameters with the data from the non-diabetic donors, we combined the results from the two groups when examining $iGABA_A$R single-channel properties and effects of days in culture on the channel properties (Figures 2 and 3).

Table 1. Cell-types identified based on expression of hormone mRNAs in pancreatic islets from non-diabetic and type 2 diabetic donors.

Cell-Type	Non-Diabetic Islets *n* Cells	Type 2 Diabetic Islets *n* Cells
α	12	3
β	65	8
δ	4	1
α/β	34	8
β/δ	18	1
α/δ	2	1
α/β/δ	14	3
Total	149	25

In rodent islets, the cell size normally correlates with the major cell-types [21,22], but the situation is somewhat different for human islet cells where we did not detect any difference in cell size among α, β and δ cells in intact islets [17]. However, it is possible that alterations in size reflect transdifferentiation of one cell-type to another. We, therefore, examined if the mixed-identity cells differed in size or if the time in culture influenced the cells' diameter. Figure 1B shows that the different subtypes of cells were similar in size, as determined from cell membrane capacitance measurements, and that the cell size

did not correlate with the time in culture after isolation of islets. We further examined if the relative expression level of a pair of hormone transcripts in the mixed-identity cells correlated with the cell size, but no correlation was found between these two parameters (Figure 1C).

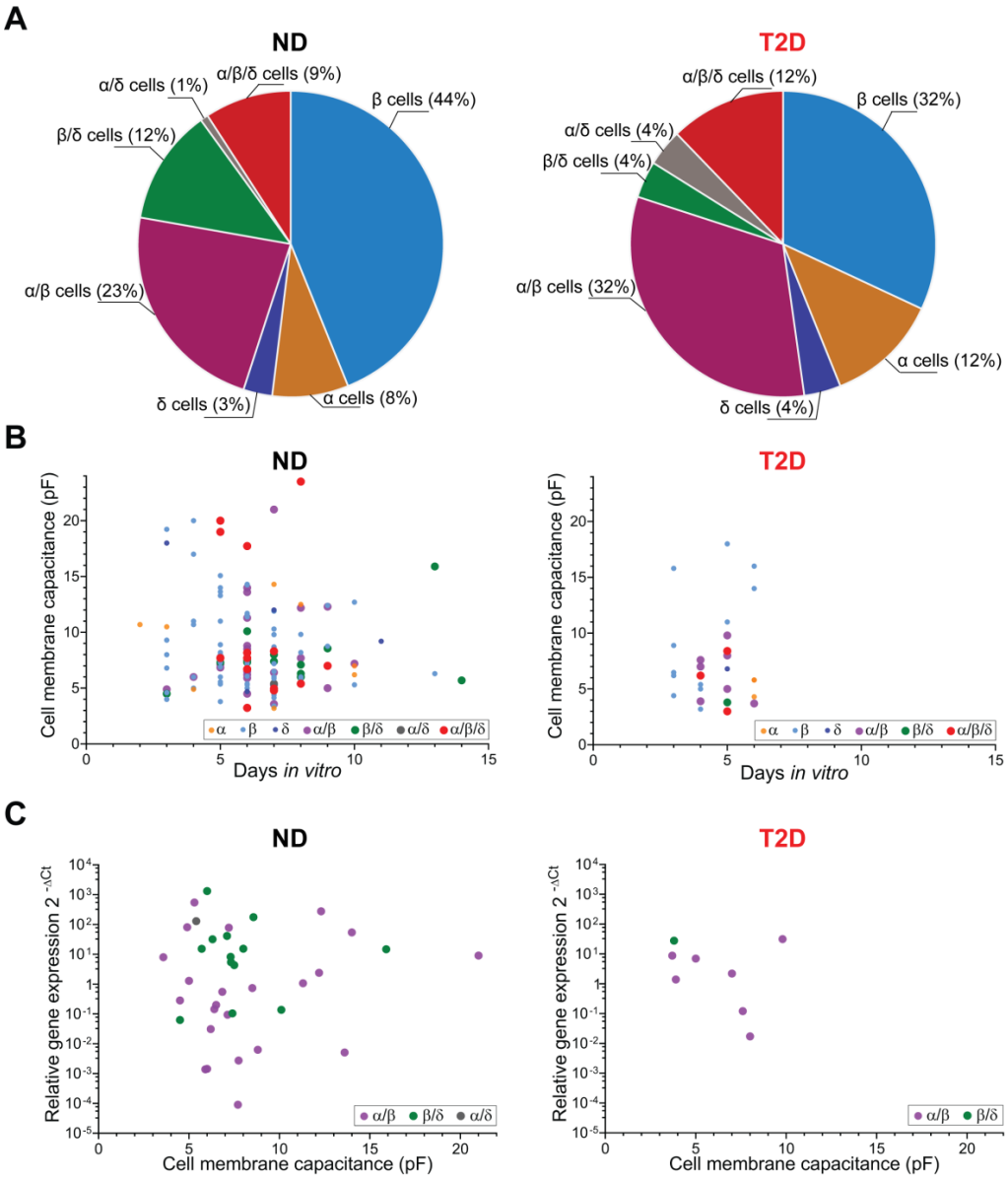

Figure 1. Percentage distribution of single and multiple hormone transcript-expressing cells (**A**) and relations between duration of islet culturing (**B**) and relative gene expression (**C**) versus cell membrane capacitance in intact human pancreatic islets from non-diabetic (ND) and type 2 diabetic (T2D) donors. Relative gene expression in (**C**) is read as the *GCG*/*INS* expression ratio for mixed-identity α/β cells (magenta circles, ND: $n = 23$, T2D: $n = 7$), *INS*/*SST* expression ratio for mixed-identity β/δ cells (green circles, ND: $n = 13$, T2D: $n = 1$) and *GCG*/*SST* expression ratio for mixed-identity α/δ cell (gray circle, ND: $n = 1$). Correlations neither in (B) (Spearman correlation coefficient for ND group r = −0.057, $p = 0.52$, $n = 130$; for T2D group r = 0.010, $p = 0.96$, $n = 27$), nor in (C) (Spearman correlation coefficient for ND group r = −0.019, $p = 0.910$, $n = 37$; for T2D group r = −0.238, $p = 0.582$, $n = 8$) are revealed. Cell membrane capacitance was measured at the holding potential, $V_h = -70$ mV. Glucose concentration in all experiments was 20 mM.

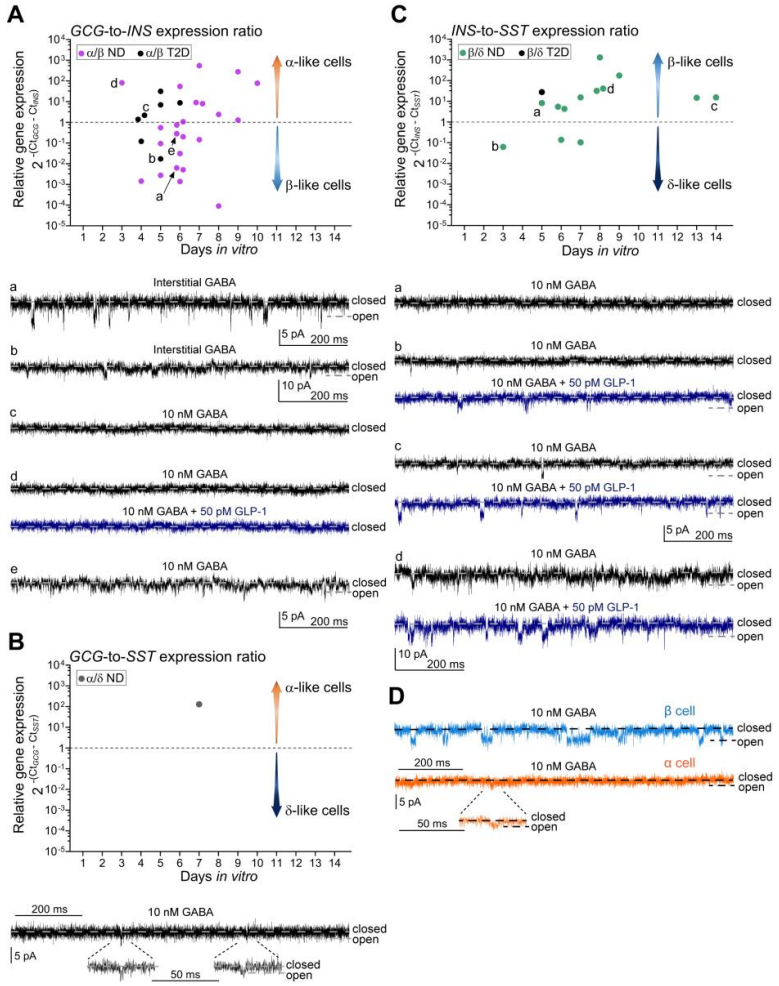

Figure 2. Ratios of hormone mRNA expressions in individual mixed-identity cells with two hormone transcripts and islet $GABA_A$ receptor ($iGABA_AR$)-mediated currents in islet cells. (**A**) The scatter dot plot of *GCG*/*INS* expression ratios in mixed-identity α/β cells and representative current recordings through $iGABA_ARs$ in α/β cells with high (**a**), medium-high (**b**), low (**d**) and comparable (**c**,**e**) levels of expression of *INS* relative to the expression level of *GCG*. Dash line at the *GCG*/*INS* expression ratio = 1 in the scatter dot plot shows equal expression of both hormone transcripts. The higher *GCG*/*INS* expression ratio, the more α/β cell is α-like (upward arrow); the lower *GCG*/*INS* expression ratio, the more α/β cell is β-like (downward arrow). (**B**) *GCG*/*SST* expression ratio in an α/δ cell and corresponding recording of $iGABA_AR$-mediated current in this cell. Two $iGABA_AR$ single-channel events with low amplitudes are shown at expanded time scale. (**C**) The scatter dot plot of *INS*/*SST* expression ratios in β/δ cells and representative current recordings (**a**–**d**) through $iGABA_ARs$ in β/δ cells. For dash line and arrows on the scatter dot plots in (**B**), (**C**) see explanations in (**A**) in context of the respective hormone transcripts. (**D**) Representative recordings showing high activity of single-channel $iGABA_ARs$ in a β cell and low activity of single-channel $iGABA_ARs$ and lower current amplitudes in an α cell in the presence of 10 nM γ-aminobutyric acid (GABA) in ND donors. A single-channel $iGABA_AR$ opening with low amplitude in the α cell is shown at expanded time scale. Closed and open states of the single channels are denoted by corresponding dash lines on the recordings in A–D. The scale bars 5 pA and 200 ms are common for the recordings Aa, c–e and Ca–c; recordings in Ab and Cd have vertical scale bar 10 pA. Recordings Ad, Cb–d were done in the presence of 10 nM GABA first (black traces), and then, 50 pM GLP-1 was added to the extracellular solution in order to examine the potentiation of $iGABA_ARs$ via the activation of GLP-1 receptor (blue traces). The recordings in Aa–b were done without exogenously added GABA (Interstitial GABA), and the rest of recordings were done in the presence of 10 nM GABA. Electrophysiological recordings were done at V_h = –70 mV. Glucose concentration in all experiments was 20 mM.

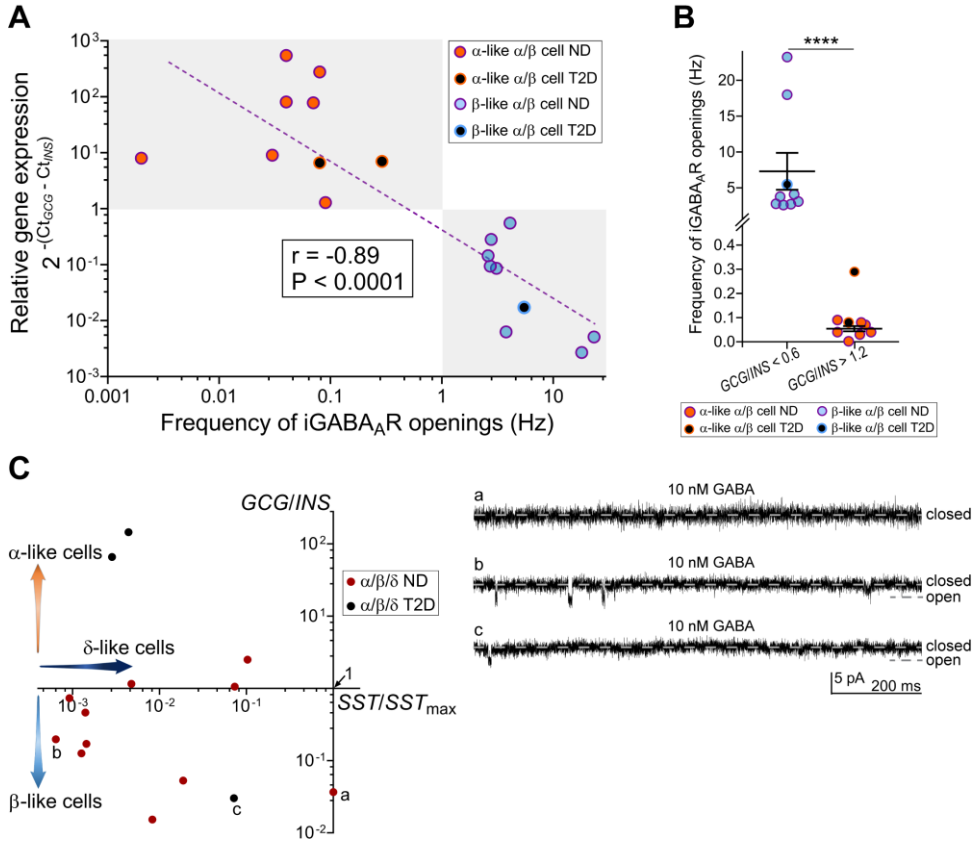

Figure 3. Frequency of single-channel iGABA$_A$R openings in mixed-identity α/β cells and combined data for mixed-identity α/β/δ cells. (**A**) Negative correlation between frequency of single-channel iGABA$_A$R openings and the relative *GCG/INS* gene expression in individual mixed-identity α/β cells showing that the higher *INS* expression in the α/β cell (= the more mixed-identity cell is a β-like), the higher frequency of the single-channel iGABA$_A$R openings in such cell. Spearman correlation coefficient r = −0.89, $p < 0.0001$, $n = 18$. (**B**) The frequency of single-channel iGABA$_A$R openings in the α/β cells with the *GCG/INS* ratio between 0.002 and 0.6 (β-like cells, see A) is significantly higher than that in α/β cells with the *GCG/INS* ratio between 1.2 and 550 (α-like cells, see A). Nonparametric Mann-Whitney test, ****$p < 0.0001$, $n = 9$ in the β-like group, $n = 8$ in the α-like group. The uppermost data point in the α-like group was obtained from T2D donor, detected as outlier by Tukey method and excluded from the comparison. (**C**) Hormone transcript expression levels and the representative iGABA$_A$R-mediated current recordings in individual mixed-identity α/β/δ cells. The hormone transcript expression levels in individual mixed-identity α/β/δ cells presented as the *GCG/INS* expression ratio in the cell versus the expression of the *SST* in the same cell normalized to the maximal expression of the *SST* among all mixed-identity α/β/δ cells (*SST*/*SST*$_{max}$). The lower *SST*/*SST*$_{max}$ ratio, the more negligible *SST* component in mixed-identity α/β/δ cell, and then the cell is considered as mixed-identity α/β cell. Thus, to be e.g., β-like, mixed-identity α/β/δ cell should fall into lower left part of the scatter plot (downward arrow). The recordings of iGABA$_A$R-mediated currents were performed from non-diabetic donors (**a**,**b**) and a type 2 diabetic donor (**c**). Recording (**a**) was obtained from the mixed-identity α/β/δ cell with the *SST*$_{max}$. Electrophysiological recordings were done at $V_h = -70$ mV. Pancreatic islets were exposed to 10 nM GABA. Glucose concentration in all experiments was 20 mM.

2.2. *iGABA$_A$R-Mediated Currents in the Different Subtypes of the Mixed-Identity Cells*

We further analysed the recordings of iGABA$_A$R-mediated currents in the mixed-identity cells in order to examine if each subtype of the mixed-identity cells had characteristic GABA-activated currents. iGABA$_A$R single-channel currents were recorded in 87% of the mixed-identity cells analysed with both electrophysiological and single-cell RT-PCR techniques. Figure 2A shows the distribution of the

GCG/*INS* expression ratio for individual mixed-identity α/β cells as a function of days in culture after the isolation of pancreas. No effect of time in culture on the *GCG*/*INS* expression ratio was detected (Spearman correlation coefficient r = 0.22, $p = 0.248$, $n = 30$ cells).

Interestingly, recordings from α/β cells with higher relative *INS* expression (corresponding to lower *GCG*/*INS* values) have higher frequency and larger amplitudes of iGABA$_A$R-mediated currents than those with higher *GCG*/*INS* expression ratio (see Figure 2Aa,b,e vs. Figure 2Ac,d). Similar results were observed for the cells with the expression ratio *GCG*/*INS* ~ 1 (Supplementary Figure S1). This is in line with the patterns of activities of iGABA$_A$Rs in single hormone transcript-expressing α and β cells [17] (see Figure 2D) and can be used to discriminate between α- and β-like α/β cells. We also examined if the frequency of single-channel openings of iGABA$_A$Rs altered with duration of the islets in culture, but no change was detected (Spearman correlation coefficient r = −0.35, $p = 0.15$, $n = 18$).

We have shown in the previous study that the activation of the glucagon-like peptide-1 receptors (GLP-1Rs) evoked prominent potentiation of the iGABA$_A$Rs in β cells [17], the cells in human pancreatic islets where the GLP-1R is most highly expressed [3,23]. The GLP-1Rs are essentially not expressed in human pancreatic islet α cells [3,23]. Accordingly, in a cell with high *GCG*/*INS* expression ratio, no potentiation of single-channel iGABA$_A$R activity with GLP-1 application was observed (Figure 2Ad) consistent with an α-like cell phenotype. Moreover, in a mixed-identity α/δ cell with high expression of *GCG* relative to *SST* (Figure 2B), we recorded low-frequency single-channel iGABA$_A$R-mediated events with low conductance that also corresponds to an α-like cell phenotype (Figure 2B,D). In the mixed-identity cells with higher *INS*/*SST* expression ratios (Figure 2C), high activity level of the single-channel events with current amplitudes comparable to those obtained in single-transcript (*INS* only) β cells were generally recorded, and the currents were potentiated by GLP-1 (Figure 2Cb–d). Nevertheless, some heterogeneity in iGABA$_A$R activity was observed (Figure 2Ca).

Detailed analysis of the data from α/β cells revealed strong negative correlation between relative *GCG*/*INS* expression levels and iGABA$_A$R single-channel opening frequency (Figure 3A; Spearman correlation coefficient r = −0.89, $p < 0.0001$, $n = 18$). Thus, the α-like α/β cells had relative expression levels of $1.2 < GCG/INS < 550$ and the β-like α/β cells of $0.002 < GCG/INS < 0.6$, and the difference in frequencies of the single-channel iGABA$_A$R openings for α-like α/β cells (0.054 ± 0.011 Hz) and for β-like α/β cells (7.30 ± 2.57 Hz) was statistically significant (mean ± SEM, nonparametric Mann–Whitney test, $p < 0.0001$, $n = 9$ in the β-like group, $n = 8$ in the α-like group; Figure 3B). We also recorded currents through iGABA$_A$Rs in mixed-identity α/β/δ cells. The most prominent iGABA$_A$R single-channel currents were recorded in cells with the highest *INS* expression among all three hormone transcripts (Figure 3Cb,c). The data in Figure 3Ca shows current recording from a mixed-identity α/β/δ cell with the maximal expression of the *SST* among all mixed-identity α/β/δ cells (SST_{max}). However, the difference in hormone transcripts expression levels varied in the mixed-identity α/β/δ cells. and the frequency of the iGABA$_A$R single-channel currents was relatively low in these cells (see Figure 3Cb,c and, e.g., Figure 2Aa,Cd).

3. Discussion

In recent years, reports have emerged indicating that there are groups of pancreatic islet cells that express more than one hormone transcript [2,3,8]. It is possible that these mixed-identity cells have properties different from single hormone transcript-expressing cells. In the current study we analysed the proportions of single hormone transcript-expressing and mixed-identity cells in islets from non-diabetic and type 2 diabetic donors and further, explored the iGABA$_A$R-mediated currents specific to a particular mixed-identity cell subtype.

Studies of type 2 diabetes have shown a decrease in the β cell mass and a concomitant augmentation in the number of α cells in islets from type 2 diabetic donors as compared to control subjects [24,25]. Our cytosome analysis corroborates these results, revealing a decreased probability of identifying single-hormone *INS*-expressing β cells in islets from type 2 diabetic donors compared to islets from non-diabetic subjects. In contrast, the probability of identifying cells containing the *GCG* increased and,

in particular, the percentage of single-hormone *GCG*-expressing α cells had a tendency for increasing in islets from type 2 diabetic donors. Whether this change is a cause or a consequence of the disease remains to be determined. No systematic change in the *GCG/INS* expression level was observed for the cells during the 10 days after isolation from the donors. Interestingly, different subtypes of the mixed-identity cells express hormone transcripts at variable levels, and several combinations exist. Apparently, the mixed-identity cells have distinct intracellular regulatory mechanisms governing particular hormone transcript expression. These cells may also differ in $iGABA_AR$ subunit composition and their expression levels that will be reflected in different patterns of $iGABA_AR$ single-channel openings.

We have previously characterized the functional properties of $iGABA_AR$ in human α and β cells [17]. Here, in mixed-identity α/β cells, we found that cells having higher *GCG/INS* expression ratio correlated with no or low $iGABA_AR$ single-channel opening frequency and low-amplitude single-channel events and no response to GLP-1 application. This pattern of activity is similar to the behaviour of $iGABA_ARs$ in single-hormone *GCG*-expressing α cells [17]. On the other hand, mixed-identity α/β cells with lower *GCG/INS* expression ratio had activity similar to single-hormone *INS*-expressing β cells [17] with higher frequency and larger amplitudes of $iGABA_AR$ single-channel openings. In the majority of the mixed-identity β/δ cells, we found the *INS* expression level was higher than that for the somatostatin transcript, and the pattern of activity of $iGABA_AR$ single channels was similar to the activity pattern recorded in the β cells. Thus, the results identify the $iGABA_AR$ single-channel currents as a functional marker of the mixed-identity cell subtype.

The explanations for the existence of mixed-identity cells in the human pancreatic islets may be many. The human islet is a plastic structure [1,26,27], and numerous factors [4,5,28], including GABA [29,30] may influence the signatures of the cells. The cell-type determination has been proposed to take place during development [31] or alter due to dedifferentiation [28] or intentional reprogramming [8]. Further studies are required to identify factors and conditions regulating the cell-type identity [32].

The GABA signalling system is an integral part of the normal human pancreatic islet physiology [15,33,34]. In particular, characteristic interstitial levels of GABA [17,19] and pulsatile nature of its release have recently been demonstrated in normally functioning islets [19]. If pancreatic islet GABA concentration changes out of the physiological range or the pulsatile GABA release is disturbed, it may impair proper insulin and glucagon secretion, potentially alter cell fate and eventually contribute to pathogenesis of type 2 diabetes [19,29,30,35]. Interstitial GABA has also been proposed to inhibit cytotoxic immune cells in the islets thus exhibiting immunomodulation relevant for both type 1 and type 2 diabetes [35–37].

In conclusion, our results show that $iGABA_AR$ single-channel activity predicts the phenotype of the mixed-identity cells. Better understanding of the effects of the GABA signalling system in the human pancreatic islets will be valuable and may assist in unravelling the relationship between the α and the β cells and, potentially, how the intrinsic potential for regeneration of the β cell mass comes about.

4. Materials and Methods

4.1. Intact Human Islets of Langerhans

The Nordic Network for Clinical Islet Transplantation generously provided human pancreatic islets. All procedures were approved by the regional ethics committee in Uppsala (Sweden). Experiments were carried out in accordance with the guidelines and regulations stipulated by appropriate Swedish and European legislation, and informed consent was obtained from donors or their relatives. The pancreata from non-diabetic and type 2 diabetic donors were treated by collagenase, and the islets were isolated by Biocoll gradient centrifugation [38]. After that, the islets were picked and cultured in CMRL 1066 (ICN Biomedicals, Costa Mesa, CA, USA) with the addition of 10 mM HEPES, 2 mM L-glutamine, 50 μg/mL gentamicin, 0.25 μg/mL fungizone (GIBCO, BRL, Gaithersburg, MD, USA),

20 μg/mL ciprofloxacin (Bayer Healthcare, Leverkusen, Germany) and 10 mM nicotinamide at 37 °C in a high-humidity atmosphere containing 5% CO_2 vol/vol and used in the experiments from the second up to the fourteenth day of culturing.

4.2. Electrophysiological Recordings

The electrophysiological recordings from cells in the superficial layers in intact islets were done in the whole-cell patch-clamp configuration at a holding potential $V_h = -70$ mV using the blind approach. The intact islet was held by the wide-bore holding pipette, and the cell within the islet was approached by the recording pipette from the opposite side. The composition of extracellular solution (in mM) was 137 NaCl, 5.6 KCl, 2.6 $CaCl_2$, 1.2 $MgCl_2$, 10 HEPES and 20 glucose (pH 7.4 using NaOH). The high glucose concentration enhances the vesicular release [39], and we used this phenomenon to maximize GABA release from the β cells and thus optimize the interstitial GABA concentration within the islets in our experiments in order to facilitate the detection of the $GABA_AR$ activity. The intracellular solution consisted of (mM): 135 CsCl, 30 CsOH, 1 $MgCl_2$, 10 EGTA, 5 HEPES and 3 Mg-ATP (pH 7.2 with HCl). Drugs were purchased from Sigma-Aldrich (Steinheim, Germany) or Ascent Scientific (Bristol, UK). Recordings were done using an Axopatch 200B amplifier, filtered at 2 kHz and digitized on-line at 10 kHz using an analog-to-digital converter. Clampex 10.5 (Molecular Devices, San Jose, CA, USA) software was used to record the original electrophysiological data. The access resistance was monitored, and if it changed by more than 25%, the recording was rejected.

Many parameters of $iGABA_ARs$ (e.g., frequency of the openings, mean open time, conductance, etc.) measured at interstitial GABA concentration are similar to values of the respective parameters obtained in the presence of 10 nM GABA (see [17] with the β cells as example). On the basis of this observation, the recordings obtained at these two GABA concentrations were considered as a common dataset when presented on Figures 2 and 3. Continuous recordings were done at interstitial GABA concentration (no GABA added to the extracellular solution) first; then, the GABA in the concentration 10 nM was added to the extracellular solution, and finally, 50 pM GLP-1 was further added to the extracellular solution in order to examine the potentiation of $iGABA_ARs$ via the activation of GLP-1 receptor, where appropriate.

4.3. Cytoplasm Harvesting and Single-Cell RT-PCR

The cytosome harvesting procedure and single-cell RT-PCR were previously described [17,21]. Briefly, after completing the patch-clamp experiment in the whole-cell configuration, the negative pressure was applied to the back of the pipette and was relieved at the moment of the whole-cell configuration destroying. Immediately after that, the pipette content was locked at the atmospheric pressure. These manipulations allowed to collect the cytosome from the cell the electrophysiological recording was done from. The pipette content (5 μL) was expelled to a 200-μL RNase-free PCR tube. The collected cytosome was subjected to the reverse transcription (RT) performed with Verso™ cDNA synthesis kit (Thermo Scientific, Waltham, MA, USA). The 20 μL of RT-reaction was exposed to 42 °C for 30 min and then incubated at 95 °C for 2 min. PCR was accomplished according to a standard procedure [17,21]. In brief, the amplification of the PCR products was done with the ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems, Waltham, MA, USA) according to the following procedure: initial denaturation step at 95 °C for 5 min; followed by 45 cycles of 95 °C for 15 s, 60 °C for 30 s and 72 °C for 1min; followed by one melting curve step. The primers for hormone transcripts are glucagon (forward: GCAACGTTCCCTTCAAGACAC, reverse: ACTGGTGAATGTGCCCTGTG), insulin (forward: CCATCAAGCAGATCACTG, reverse: CACTAGGTAGAGAGCTTCC) and somatostatin (forward: CCCAGACTCCGTCAGTTTCT, reverse: AAGTACTTGGCCAGTTCCTGC). The efficiency of primers for each hormone transcript was in the range between 99 and 100%. The relative expression of pairs of hormone transcripts (mRNA) in individual mixed-identity cells was defined as $2^{-(Ct(mRNA1)-Ct(mRNA2))}$. The melting curve of the PCR product was examined and/or PCR product was run on a 1.5% agarose gel. RNA from whole human

islet samples and the intracellular solution or water served as the positive control and negative control, respectively.

4.4. Data Analysis

Statistical dependences between different parameters measured in electrophysiological or single-cell RT-PCR experiments were tested by Spearman correlation using GraphPad Prism 7 (La Jolla, CA, USA). The Tukey method was used for the detection of outliers which were excluded from the analysis. Nonparametric Mann–Whitney test was used to compare groups which contained not normally distributed data. Significance level was set at $p < 0.05$. The values are mean ± SEM.

5. Patents

Bryndis Birnir has filed two patent applications (1850201-3, 1850211-2) based on GABA and $GABA_A$ receptors function.

Supplementary Materials: Supplementary materials can be found at http://www.mdpi.com/1422-0067/21/2/600/s1.

Author Contributions: Conceptualization, S.V.K., Z.J. and B.B.; formal analysis, S.V.K and Z.J.; funding acquisition, S.V.K. and B.B.; investigation, S.V.K. and Z.J.; visualization, S.V.K.; writing—original draft, S.V.K. and B.B. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: B.B. has filed two patent applications (1850201-3, 1850211-2) based on GABA and $GABA_A$ receptors function. S.V.K. and Z.J. have no conflict of interests to disclose. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript or in the decision to publish the results.

Abbreviations

GCG	gene coding the glucagon protein (GCG)
GLP-1R	glucagon-like peptide-1 receptor
$iGABA_AR$	islet γ-aminobutyric acid receptor type A
INS	gene coding the insulin protein (INS)
ND	no diabetes
RT-PCR	reverse transcription polymerase chain reaction
SST	gene coding the somatostatin protein (SST)
T2D	type 2 diabetes
V_h	holding potential

References

1. Steiner, D.J.; Kim, A.; Miller, K.; Hara, M. Pancreatic islet plasticity: Interspecies comparison of islet architecture and composition. *Islets* **2010**, *2*, 135–145. [CrossRef] [PubMed]
2. Muraro, M.J.; Dharmadhikari, G.; Grün, D.; Groen, N.; Dielen, T.; Jansen, E.; van Gurp, L.; Engelse, M.A.; Carlotti, F.; de Koning, E.J.; et al. A Single-Cell Transcriptome Atlas of the Human Pancreas. *Cell Syst.* **2016**, *3*, 385–394. [CrossRef] [PubMed]
3. Segerstolpe, Å.; Palasantza, A.; Eliasson, P.; Andersson, E.M.; Andréasson, A.C.; Sun, X.; Picelli, S.; Sabirsh, A.; Clausen, M.; Bjursell, M.K.; et al. Single-Cell Transcriptome Profiling of Human Pancreatic Islets in Health and Type 2 Diabetes. *Cell Metab.* **2016**, *24*, 593–607. [CrossRef] [PubMed]

4. White, M.G.; Marshall, H.L.; Rigby, R.; Huang, G.C.; Amer, A.; Booth, T.; White, S.; Shaw, J.A. Expression of mesenchymal and α-cell phenotypic markers in islet β-cells in recently diagnosed diabetes. *Diabetes Care* **2013**, *36*, 3818–3820. [CrossRef] [PubMed]
5. Sun, J.; Ni, Q.; Xie, J.; Xu, M.; Zhang, J.; Kuang, J.; Wang, Y.; Ning, G.; Wang, Q. Beta cell dedifferentiation in T2D patients with adequate glucose control and non-diabetic chronic pancreatitis. *J. Clin. Endocrinol. Metab.* **2018**, *104*, 83–94. [CrossRef] [PubMed]
6. Cigliola, V.; Thorel, F.; Chera, S.; Herrera, P.L. Stress-induced adaptive islet cell identity changes. *Diabetes Obes. Metab.* **2016**, *18* (Suppl. 1), 87–96. [CrossRef]
7. Moin, A.S.M.; Butler, A.E. Alterations in Beta Cell Identity in Type 1 and Type 2 Diabetes. *Curr. Diab. Rep.* **2019**, *19*, 83. [CrossRef]
8. Kordowich, S.; Mansouri, A.; Collombat, P. Reprogramming into pancreatic endocrine cells based on developmental cues. *Mol. Cell Endocrinol.* **2010**, *315*, 11–18. [CrossRef]
9. Wang, Z.; York, N.W.; Nichols, C.G.; Remedi, M.S. Pancreatic β cell dedifferentiation in diabetes and redifferentiation following insulin therapy. *Cell Metab.* **2014**, *19*, 872–882. [CrossRef]
10. Ramracheya, R.; Ward, C.; Shigeto, M.; Walker, J.N.; Amisten, S.; Zhang, Q.; Johnson, P.R.; Rorsman, P.; Braun, M. Membrane potential-dependent inactivation of voltage-gated ion channels in alpha-cells inhibits glucagon secretion from human islets. *Diabetes* **2010**, *59*, 2198–2208. [CrossRef]
11. Braun, M.; Ramracheya, R.; Bengtsson, M.; Zhang, Q.; Karanauskaite, J.; Partridge, C.; Johnson, P.R.; Rorsman, P. Voltage-gated ion channels in human pancreatic beta-cells: Electrophysiological characterization and role in insulin secretion. *Diabetes* **2008**, *57*, 1618–1628. [CrossRef] [PubMed]
12. Braun, M.; Ramracheya, R.; Amisten, S.; Bengtsson, M.; Moritoh, Y.; Zhang, Q.; Johnson, P.R.; Rorsman, P. Somatostatin release, electrical activity, membrane currents and exocytosis in human pancreatic delta cells. *Diabetologia* **2009**, *52*, 1566–1578. [CrossRef] [PubMed]
13. Rorsman, P.; Berggren, P.O.; Bokvist, K.; Ericson, H.; Möhler, H.; Ostenson, C.G.; Smith, P.A. Glucose-inhibition of glucagon secretion involves activation of GABAA-receptor chloride channels. *Nature* **1989**, *341*, 233–236. [CrossRef] [PubMed]
14. Wendt, A.; Birnir, B.; Buschard, K.; Gromada, J.; Salehi, A.; Sewing, S.; Rorsman, P.; Braun, M. Glucose inhibition of glucagon secretion from rat alpha-cells is mediated by GABA released from neighboring beta-cells. *Diabetes* **2004**, *53*, 1038–1045. [CrossRef] [PubMed]
15. Braun, M.; Ramracheya, R.; Bengtsson, M.; Clark, A.; Walker, J.N.; Johnson, P.R.; Rorsman, P. Gamma-aminobutyric acid (GABA) is an autocrine excitatory transmitter in human pancreatic beta-cells. *Diabetes* **2010**, *59*, 1694–1701. [CrossRef] [PubMed]
16. Taneera, J.; Jin, Z.; Jin, Y.; Muhammed, S.J.; Zhang, E.; Lang, S.; Salehi, A.; Korsgren, O.; Renström, E.; Groop, L.; et al. γ-Aminobutyric acid (GABA) signalling in human pancreatic islets is altered in type 2 diabetes. *Diabetologia* **2012**, *55*, 1985–1994. [CrossRef]
17. Korol, S.V.; Jin, Z.; Jin, Y.; Bhandage, A.K.; Tengholm, A.; Gandasi, N.R.; Barg, S.; Espes, D.; Carlsson, P.O.; Laver, D.; et al. Functional Characterization of Native, High-Affinity GABAA Receptors in Human Pancreatic β Cells. *EBioMedicine* **2018**, *30*, 273–282. [CrossRef]
18. Untereiner, A.; Abdo, S.; Bhattacharjee, A.; Gohil, H.; Pourasgari, F.; Ibeh, N.; Lai, M.; Batchuluun, B.; Wong, A.; Khuu, N.; et al. GABA promotes β-cell proliferation, but does not overcome impaired glucose homeostasis associated with diet-induced obesity. *FASEB J.* **2019**, *33*, 3968–3984. [CrossRef]
19. Menegaz, D.; Hagan, D.W.; Almaça, J.; Cianciaruso, C.; Rodriguez-Diaz, R.; Molina, J.; Dolan, R.M.; Becker, M.W.; Schwalie, P.C.; Nano, R.; et al. Mechanism and effects of pulsatile GABA secretion from cytosolic pools in the human beta cell. *Nat. Metab.* **2019**, *1*, 1110–1126. [CrossRef]
20. Tian, J.; Dang, H.; Chen, Z.; Guan, A.; Jin, Y.; Atkinson, M.A.; Kaufman, D.L. γ-Aminobutyric acid regulates both the survival and replication of human β-cells. *Diabetes* **2013**, *62*, 3760–3765. [CrossRef]
21. Jin, Y.; Korol, S.V.; Jin, Z.; Barg, S.; Birnir, B. In intact islets interstitial GABA activates GABA(A) receptors that generate tonic currents in α-cells. *PLoS ONE* **2013**, *8*, e67228. [CrossRef] [PubMed]
22. Briant, L.J.; Zhang, Q.; Vergari, E.; Kellard, J.A.; Rodriguez, B.; Ashcroft, F.M.; Rorsman, P. Functional identification of islet cell types by electrophysiological fingerprinting. *J. R. Soc. Interface* **2017**, *14*, 20160999. [CrossRef] [PubMed]
23. Tornehave, D.; Kristensen, P.; Rømer, J.; Knudsen, L.B.; Heller, R.S. Expression of the GLP-1 receptor in mouse, rat, and human pancreas. *J. Histochem. Cytochem.* **2008**, *56*, 841–851. [CrossRef]

24. Yoon, K.H.; Ko, S.H.; Cho, J.H.; Lee, J.M.; Ahn, Y.B.; Song, K.H.; Yoo, S.J.; Kang, M.I.; Cha, B.Y.; Lee, K.W.; et al. Selective beta-cell loss and alpha-cell expansion in patients with type 2 diabetes mellitus in Korea. *J. Clin. Endocrinol. Metab.* **2003**, *88*, 2300–2308. [CrossRef] [PubMed]
25. Deng, S.; Vatamaniuk, M.; Huang, X.; Doliba, N.; Lian, M.M.; Frank, A.; Velidedeoglu, E.; Desai, N.M.; Koeberlein, B.; Wolf, B.; et al. Structural and functional abnormalities in the islets isolated from type 2 diabetic subjects. *Diabetes* **2004**, *53*, 624–632. [CrossRef] [PubMed]
26. Dorrell, C.; Schug, J.; Canaday, P.S.; Russ, H.A.; Tarlow, B.D.; Grompe, M.T.; Horton, T.; Hebrok, M.; Streeter, P.R.; Kaestner, K.H.; et al. Human islets contain four distinct subtypes of β cells. *Nat. Commun.* **2016**, *7*, 11756. [CrossRef] [PubMed]
27. Wang, Y.J.; Golson, M.L.; Schug, J.; Traum, D.; Liu, C.; Vivek, K.; Dorrell, C.; Naji, A.; Powers, A.C.; Chang, K.M.; et al. Single-Cell Mass Cytometry Analysis of the Human Endocrine Pancreas. *Cell Metab.* **2016**, *24*, 616–626. [CrossRef]
28. Teo, A.K.K.; Lim, C.S.; Cheow, L.F.; Kin, T.; Shapiro, J.A.; Kang, N.Y.; Burkholder, W.; Lau, H.H. Single-cell analyses of human islet cells reveal de-differentiation signatures. *Cell Death Discov.* **2018**, *4*, 14. [CrossRef]
29. Ben-Othman, N.; Vieira, A.; Courtney, M.; Record, F.; Gjernes, E.; Avolio, F.; Hadzic, B.; Druelle, N.; Napolitano, T.; Navarro-Sanz, S.; et al. Long-Term GABA Administration Induces Alpha Cell-Mediated Beta-like Cell Neogenesis. *Cell* **2017**, *168*, 73–85. [CrossRef]
30. Li, J.; Casteels, T.; Frogne, T.; Ingvorsen, C.; Honoré, C.; Courtney, M.; Huber, K.V.M.; Schmitner, N.; Kimmel, R.A.; Romanov, R.A.; et al. Artemisinins Target GABAA Receptor Signaling and Impair Alpha Cell Identity. *Cell* **2017**, *168*, 86–100. [CrossRef]
31. Riedel, M.J.; Asadi, A.; Wang, R.; Ao, Z.; Warnock, G.L.; Kieffer, T.J. Immunohistochemical characterisation of cells co-producing insulin and glucagon in the developing human pancreas. *Diabetologia* **2012**, *55*, 372–381. [CrossRef] [PubMed]
32. Furuyama, K.; Chera, S.; van Gurp, L.; Oropeza, D.; Ghila, L.; Damond, N.; Vethe, H.; Paulo, J.A.; Joosten, A.M.; Berney, T.; et al. Diabetes relief in mice by glucose-sensing insulin-secreting human α-cells. *Nature* **2019**, *567*, 43–48. [CrossRef] [PubMed]
33. Caicedo, A. Paracrine and autocrine interactions in the human islet: More than meets the eye. *Semin. Cell Dev. Biol.* **2013**, *24*, 11–21. [CrossRef] [PubMed]
34. Wang, Q.; Ren, L.; Wan, Y.; Prud'homme, G.J. GABAergic regulation of pancreatic islet cells: Physiology and antidiabetic effects. *J. Cell Physiol.* **2019**, *234*, 14432–14444. [CrossRef] [PubMed]
35. Soltani, N.; Qiu, H.; Aleksic, M.; Glinka, Y.; Zhao, F.; Liu, R.; Li, Y.; Zhang, N.; Chakrabarti, R.; Ng, T.; et al. GABA exerts protective and regenerative effects on islet beta cells and reverses diabetes. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 11692–11697. [CrossRef]
36. Bjurstöm, H.; Wang, J.; Ericsson, I.; Bengtsson, M.; Liu, Y.; Kumar-Mendu, S.; Issazadeh-Navikas, S.; Birnir, B. GABA, a natural immunomodulator of T lymphocytes. *J. Neuroimmunol.* **2008**, *205*, 44–50. [CrossRef]
37. Bhandage, A.K.; Jin, Z.; Korol, S.V.; Shen, Q.; Pei, Y.; Deng, Q.; Espes, D.; Carlsson, P.O.; Kamali-Moghaddam, M.; Birnir, B. GABA Regulates Release of Inflammatory Cytokines From Peripheral Blood Mononuclear Cells and CD4+ T Cells and Is Immunosuppressive in Type 1 Diabetes. *EBioMedicine* **2018**, *30*, 283–294. [CrossRef]
38. Fred, R.G.; Bang-Berthelsen, C.H.; Mandrup-Poulsen, T.; Grunnet, L.G.; Welsh, N. High glucose suppresses human islet insulin biosynthesis by inducing miR-133a leading to decreased polypyrimidine tract binding protein-expression. *PLoS ONE* **2010**, *5*, e10843. [CrossRef]
39. Braun, M.; Wendt, A.; Birnir, B.; Broman, J.; Eliasson, L.; Galvanovskis, J.; Gromada, J.; Mulder, H.; Rorsman, P. Regulated exocytosis of GABA-containing synaptic-like microvesicles in pancreatic beta-cells. *J. Gen. Physiol.* **2004**, *123*, 191–204. [CrossRef]