Electrophysiological properties of human β -cell lines EndoC- β H1 and - β H2 conform with human β -cells

Short running title (47 characters and spaces): *Characterization of human β -cell lines*

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Abstract

The electrophysiological and secretory properties of the human β -cell lines EndoC- β H1 and EndoC- β H2 were investigated. Both cell lines respond to glucose (6-20mM) with 2- to 3-fold stimulation of insulin secretion, an effect that was mimicked by tolbutamide (0.2mM) and reversed by diazoxide (0.5mM). Glucose-induced insulin release correlated with an elevation of $[Ca^{2+}]_i$, membrane depolarization and increased action potential firing. K_{ATP} channel activity at 1mM glucose is low and increasing glucose to 6 or 20mM reduced K_{ATP} channel activity to the same extent as application of the K_{ATP} channel blocker tolbutamide (0.2mM). The upstroke of the action potentials in EndoC- β H1 and - β H2 cells observed at high glucose principally reflects activation of L- and P/Q-type Ca^{2+} channels with some small contribution of TTX-sensitive Na^+ channels. Action potential repolarization involves activation of voltage-gated $Kv2.2$ channels and large-conductance Ca^{2+} -activated K^+ channels. Exocytosis (measured by measurements of membrane capacitance) was triggered by membrane depolarizations $>10ms$ to membrane potentials above $-30mV$. Both cell lines were well-granulated (6,000-15,000 granules/cell) and granules consisted of a central insulin core surrounded by a clear halo. We conclude that the EndoC- β H1 and - β H2 cells share many features of primary human β -cells and that they represent a useful experimental model.

Introduction

Electrical activity plays a critical role in glucose-stimulated insulin secretion (GSIS) (1, 2). Understanding of the stimulus-secretion coupling in β -cells is important as its dysfunction is recognized to be central in type 2 diabetes. Indeed, the majority of loci associated with diabetes risk by genome-wide association studies (GWAS) affect β -cell function and/or mass (3-6). However, exactly how these variants impact on β -cell function has only been established for a few of them.

The limited availability of human islets preparations and the donor variability have hampered the study of human β -cell function. Consequently, how genetic variants affect β -cell function remains a challenging topic to explore. It is clear that access to a human β -cell line amenable to genetic modification would be extremely valuable. The EndoC- β H1 and - β H2 cells were generated from human fetal pancreatic bud and express numerous β -cell markers. These human β -cell lines respond to elevated glucose with stimulation of insulin secretion (7, 8) and are increasingly used to explore various aspects of human β -cell biology (9-20). Here, we monitored different parameters that constitute the triggering pathway of the GSIS (1, 21), the electrophysiological and ultrastructural properties of EndoC- β H1 and - β H2 cells. We correlate our functional findings to transcript levels determined by RNA sequencing. Overall, our data show consistency between the EndoC- β H1 and - β H2 cells and primary human β -cells.

Research Design and Methods

All procedures summarized below are reported in detail in the Supplementary data section.

Cell lines and cell culture and insulin secretion

EndoC- β H1 and - β H2 cell lines were cultured as described in the original reports (7, 8). Insulin secretion assays were performed on cells cultured on coated 24 wells plates (Nunc Life Technologies Ltd, Paisley, UK) and adapted from protocols previously described (7, 8, 22). The somatostatin receptor 2 (SSTR2) antagonist CYN154806 (cat. No. 1843) was obtained from TOCRIS.

Electrophysiology

All electrophysiological experiments were performed as described previously (23). Agatoxin, isradipine, SNX 482, stromtoxin and iberiotoxin were purchased from Alomone and tetrodotoxin (TTX) from Sigma.

[Ca²⁺]_i imaging

EndoC- β H1 and - β H2 cells were transfected with GCaMP5G (Addgene plasmid, # 31788) using lipofectamine (ThermoFischer Scientific). Transfected cells were imaged using a 10-14x magnification on a Zeiss AxioZoom.V16 microscope (Zeiss, Germany).

Immunocytochemistry and electron microscopy

Insulin, glucagon, and somatostatin were detected using the following antibodies: guinea-pig anti-insulin (in house), mouse anti-glucagon (Sigma, Gillingham, UK) and mouse anti-somatostatin (Santa Cruz Biotechnology, Germany). For electron microscopy, the cells were fixed in 2.5% glutaraldehyde and embedded in London Resin Gold (Agar Scientific). Ultrathin sections were immunogold-labeled for insulin, glucagon and somatostatin. Sections were viewed on a Joel 1010 microscope with a digital camera (Gatan, Abingdon).

RNA sequencing

RNA was extracted from EndoC- β H1 and EndoC- β H2 cell lines using TRIzol and sequenced at the Oxford Genomics Centre (Wellcome Centre for Human Genetics, University of Oxford) (24, 25). These data are deposited at the European Nucleotide Archive (<https://www.ebi.ac.uk/ena>) under the accession number PRJEB23293 (the transcriptome analysis can also be found in Supplementary table 1).

Data analysis

Data are presented as mean $\pm$ S.E.M. Statistical significances were evaluated as detailed in the Figure legends.

Results

Glucose-induced insulin release in EndoC- β H1 and - β H2 cells

Insulin secretion at 1mM glucose (normalized to insulin content) was ~4.5% per hour (/h) and ~3%/h in EndoC- β H1 and - β H2 cells, respectively (Fig. 1A). Increasing glucose to 6mM, stimulated insulin secretion by 3-fold in EndoC- β H1 and by 1.5-fold in the EndoC- β H2 cells (Fig. 1B). Whereas elevating glucose to 20mM had no additive effect in EndoC- β H1, it resulted in a further 50% stimulation in EndoC- β H2 cells. In both cell types, the stimulatory effect of glucose was prevented by the K_{ATP} channel activator diazoxide (0.5mM) and mimicked (in part) by the K_{ATP} channel blocker tolbutamide (0.2mM) (Fig. 1B).

Transcriptomic analysis of these cell lines obtained by RNA-sequencing revealed that key genes involved in the β -cell glucose sensitivity were also expressed in these cell

lines. The K_{ATP} channels subunits *KCNJ11* (Kir6.2) and *ABCC8* (SUR1) as well as *SLC2A1* (encoding for the glucose transporter GLUT1) and *GCK* (glucokinase) are expressed at comparable levels in EndoC- β H1 and - β H2 (Supplementary Fig. 1 and Supplementary Table1). Whereas *SLC2A2* (GLUT2) is expressed at levels approaching *SLC2A1* in EndoC- β H1 cells, its expression is very low in EndoC- β H2 cells (Supplementary Fig. 1 and Supplementary Table 1).

Glucose triggers increases in $[Ca^{2+}]_i$ in EndoC- β H1 and - β H2 cells

Insulin is secreted in response to an elevation in cytoplasmic Ca^{2+} ($[Ca^{2+}]_i$). We correlated the insulin secretion data to changes in $[Ca^{2+}]_i$ in EndoC- β H1 and - β H2 cells transfected with the genetically encoded Ca^{2+} indicator GCaMP5 (Fig. 1C). The heatmaps below the traces (GCaMP5 fluorescence normalized to basal fluorescence; F/F_0) illustrate the substantial heterogeneity between cells. Basal activity was higher in EndoC- β H1 than in EndoC- β H2 cells. Spontaneously active cells responded poorly to both high glucose and tolbutamide (bottom part of the heatmap). Yet and despite the variability, stimulation with 6 and 20mM glucose or 200 μ M tolbutamide increases $[Ca^{2+}]_i$ significantly (Fig. 1D).

EndoC- β H1 and - β H2 cells are electrically excitable

We correlated insulin secretion and changes in $[Ca^{2+}]_i$ to electrical activity. At 1mM glucose, the membrane potential was -70 to -60mV and many EndoC- β H1 and - β H2-cells exhibited spontaneous action potential firing. Increasing glucose to 20mM resulted in membrane depolarization to -55mV and stimulation of electrical activity (Fig. 2A). On average, glucose (20mM) increased action potential frequency by 30- and 25-fold in EndoC- β H1 and - β H2 cells, respectively (Fig. 2B). Fig. 2C compares

the characteristics of glucose-induced action potentials in EndoC- β H1 and - β H2 cells with those in primary human β -cells. In primary β -cells and EndoC- β H2, the action potential peaked at 5mV. However, in EndoC- β H1 cells, the action potentials were broader (with variable duration) and in 90% of the cells they peaked at voltages above +5mV.

The resting membrane conductance (G_m) averaged 50-60pS/pF in both cell types (Fig. 2D). This is in good agreement with that measured in primary human β -cells (1). High glucose and tolbutamide reduced G_m by $\approx$ 50% (Fig. 2E). Conversely, addition of the K_{ATP} channel activator diazoxide (0.5mM) in the presence of 20mM glucose increased by 200-400%. The average cell capacitance (proportional to the cell size) is of 10.6pF and 12.5 pF for EndoC- β H1 and - β H2 respectively (Fig. 2F).

We next characterized the voltage-gated membrane currents that underlie action potentials.

Voltage-gated Na^+ channels

Voltage-gated Na^+ currents (I_{Na}) elicited by membrane depolarizations from -70 to 0mV in EndoC- β H1 and EndoC- β H2 cells were insensitive to the Ca^{2+} channel blocker Co^{2+} but highly blocked by tetrodotoxin (TTX) (Fig. 3A). Fig. 3B shows families of Na^+ currents during depolarizations between -60 and +20mV evoked from a holding potential of -150mV or -70mV. Both activation and inactivation became more rapid with stronger depolarizations. At 0mV, activation and inactivation was complete within <1ms and 5ms, respectively. Fig. 3C shows the current (I)-voltage (V) relationships recorded from EndoC- β H1 and - β H2 cells when the cells were held at either -70mV

or -150mV. In both cell types, I_{Na} became detectable during depolarizations to -40mV, was maximal at 0 mV and decreased at more positive voltages with an extrapolated reversal potential at +70mV. The maximum current elicited from -70mV (-9.5 ± 1 pA/pF, $n=24$ EndoC- β H1 cells and -19 ± 7 pA/pF, $n=11$ EndoC- β H2 cells) increased by 90% and 60% respectively when the cells were held at -150mV (Fig. 3C).

We described the voltage dependence of activation from -70mV by fitting the I-V relationship to Eq. 1 (see Supplementary material). Activation of I_{Na} was half-maximal ($V_{0.5}$) at -18 ± 0.3 mV ($n=26$) and -19 ± 0.4 mV ($n=23$) in EndoC- β H1 and EndoC- β H2 cells, respectively (Fig. 3D).

Voltage-gated Na^+ channels characteristically undergo voltage-dependent inactivation. We characterized this by a two-pulse protocol (a standard test pulse was preceded by a conditioning prepulse). In EndoC- β H1 and - β H2 cells 58% and 36% of the measured I_{Na} exhibited monophasic inactivation; half-maximal inactivation (V_h ; see Eq. 2 in Supplementary material) was at -63 ± 2 mV ($n=15$) and -59 ± 2 mV ($n=12$) in EndoC- β H1 and - β H2 cells, respectively (Fig. 3E). The remaining cells showed biphasic voltage dependence of inactivation and in addition to the main component contained a current component that accounted for 30-40% of the total current that inactivated with V_h -values of -94 ± 4 mV ($n=11$) to -91 ± 3 mV ($n=21$) (not shown). The latter component likely accounts for the increase in I_{Na} amplitude when the cells were held at -150mV rather than -70mV but its functional significance remains obscure as it will be completely inactivated at physiological membrane potentials. Overall, the I_{Na} activation and inactivation properties in EndoC- β H1 and - β H2 cells are similar to those observed in primary human β -cells (23, 26).

Fig. 2C shows I_{Na} during an action potential-like voltage-clamp stimulation (based on the action potentials recorded in EndoC- β H2 cells). Peak I_{Na} elicited by this stimulation paradigm were small: $3.1 \pm 0.3 \text{ pA.pF}^{-1}$ and $3.9 \pm 1 \text{ pA.pF}^{-1}$ in EndoC- β H1 and - β H2 cells, respectively (Fig. 3F).

At the mRNA level and compared to the set of Na^+ channels transcripts EndoC- β H1 and - β H2 cells express particularly high levels of *SNC8A* ($\text{Na}_v1.6$) and *SNC9A* ($\text{Na}_v1.7$). *SCN3A* ($\text{Nav}1.3$) is also highly expressed in EndoC- β H2 cells. In addition, *SCN5A* and *SCN7A* are expressed at relatively high levels but these encode TTX-resistant channels. Given that I_{Na} in both cell types is TTX-sensitive, it appears that these transcripts do not give rise to functional channels. Of the β -subunits, only *SCN3B* is expressed at significant levels (Supplementary Fig. 2, and Supplementary Table 1).

Voltage-gated Ca^{2+} channels

Voltage-gated Ca^{2+} currents (I_{Ca}) were recorded in the presence of TTX. The relative contribution of P/Q-, L- and R-type Ca^{2+} channels was estimated by sequential addition of ω -agatoxin, isradipine and SNX482 (Fig. 4A,C). Fig. 4B, D compares the voltage dependence of the total I_{Ca} and the different (pharmacologically isolated) I_{Ca} components. In both EndoC- β H1 and - β H2 cells, there was a ‘shoulder’ at voltages between -50 and -20mV. It arises because the L-type Ca^{2+} channels activate at slightly more negative voltages than the P/Q- and R-type Ca^{2+} channels (peak currents at -20 to 0mV vs +10mV). In both cell types, P/Q-type Ca^{2+} channels accounted for ~60% of the total current whereas L- and R-type Ca^{2+} channels contributed 20-30% and 5-10%, respectively (Fig. 4B, D). Similar to previous measurement on primary human β -cell

(23), the total I_{Ca} density was ~ 10 pA/pF in both cell types (Fig. 4E). Fig. 4G compares the activation of the total I_{Ca} and the respective components during an action potential in EndoC- β H2 cells. It is clear that both the L- and P/Q-type Ca^{2+} channels activate during the brief action potential.

Consistent with the electrophysiology, RNA-seq analyses revealed high expression of *CACNA1A* (P/Q-type) and *CACNA1C/D* (L-type) Ca^{2+} channels and low levels of expression of *CACNA1E* (R-type). In addition, *CACNA1H* (T-type) and *CACNA1B* (N-type) Ca^{2+} channels were expressed at high levels although current components corresponding to these channels could not be isolated by the pharmacological approaches. Of the auxiliary subunits, high expression of *CACNG4* (γ_4), *CACNA2D* ($\alpha_2\delta_2$) and *CACNB1-3* (β_1 - β_3) was observed (Supplementary Fig. 3; and Supplementary Table 1).

Voltage-gated K^+ channels

Voltage-activated K^+ -currents (I_K) elicited during membrane depolarizations from -70mV to membrane potentials between -20 and +70mV in EndoC- β H1 and - β H2 cells are shown in Fig. 5A. The amplitude of I_K increased linearly with the applied voltage and the density was $\sim 50\%$ larger in EndoC- β H2 than in - β H1 cells (Fig. 5B). We estimated the contribution of voltage- (K_v) and Ca^{2+} -activated (BK) channels to I_K by sequential addition of stromatoxin and iberiotoxin (Fig. 5C). Notably, BK channels played a more prominent role in EndoC- β H2 than in EndoC- β H1 during depolarizations to -10 and 0mV (Fig. 5D). Fig. 5E shows the contribution of K_v and BK channels to the outward current during voltage-clamp depolarizations based on the action potentials in EndoC- β H1 and - β H2 cells. This analysis reveals that BK current component

activated rapidly during the action potential and at time when no outward current was seen in the EndoC- β H1 cells (Fig. 5E, vertical dashed line).

At the mRNA level, EndoC- β H1 and - β H2 cells express particularly high levels of the K_v channel genes *KCNH6*, *H2*, *KCNB2*, *KCNQ2* and the BK channel genes *KCNMA1* and *KCNMB3* (Supplementary Fig. 4 and Supplementary Table 1).

Exocytosis

Depolarization-evoked exocytosis was monitored as increases in cell capacitance. Fig. 6A-B compares the voltage dependence of exocytosis in EndoC- β H1 and - β H2 cells. Exocytosis was triggered by 500ms depolarizations to voltages between -40 and +40mV. In both cell types, exocytosis was elicited at voltages above -30mV, were maximal at ~0mV and declined at more depolarized voltages. This voltage dependence echoes that of I_{Ca} . The kinetics of exocytosis during depolarizations to 0mV (i.e. close to the peak of the action potential) was determined by application of progressively longer depolarizations (Fig. 6C-D). In both EndoC- β H1 and - β H2 cells, exocytosis was elicited by depolarizations as short as 20ms, showed an initial plateau between 50 and 100ms and a secondary acceleration during longer depolarizations. The exocytotic response to a 800ms depolarization at steady-state was 119 ± 31 fF and 181 ± 40 fF in EndoC- β H1 and - β H2 respectively (Fig. 6D, insert).

We also monitored exocytosis during repetitive stimulation consisting of ten 500ms depolarizations to 0mV (Fig. 6E). Fig. 6F compares the responses to the individual depolarizations in EndoC- β H1 and - β H2 cells. The kinetics of exocytosis was biphasic echoing the kinetics observed in primary human β -cells (27). The largest response was

elicited by the initial depolarizations with the first two pulses accounting for $\geq 50\%$ of the total increase in capacitance and the responses during the last four pulses were small. The total increase in cell capacitance evoked by the train averaged 450fF in both cell types (Fig. 6F, inset).

Gene transcription analysis of proteins involved in exocytosis revealed higher expression of *SNAP25* in EndoC- β H1, than in EndoC- β H2 cells. The other SNAREs, *VAMP2* and Syntaxin1A (*STX1A*) were expressed to the same extent (Supplementary Fig. 5 and Supplementary Table 1). Other components of the exocytotic machinery, including munc18a-c (*STXBPI-3*), munc13b (*UNC13B*) and the complexins (*CPLX1-2*) were also highly expressed. Of the Ca^{2+} sensors of exocytosis, the high-affinity Ca^{2+} sensor *SYT7* was the predominant synaptotagmin in both cell types but relatively high levels of *SYT1*, 5 and 9 were also detected in EndoC- β H1 cells.

Subset of EndoC- β H1 and - β H2 cells are polyhormonal cells.

Ultrastructural analysis revealed that the secretory granules in EndoC- β H1 and - β H2 were of variable size and that most granules exhibited a clear thin halo and a central insulin-containing core (Fig. 7A). The granule density (granules per cytoplasm area; N_A) was slightly higher in EndoC- β H2 cells than in the - β H1 cells (Fig. 7B) but the intragranular insulin density (estimated from immunogold labelling) was correspondingly reduced (Fig. 7C). In both cell types, the cross-sectional granule area was $0.03\mu\text{m}^2$ (Fig. 7D), corresponding to a granule diameter of 200nm from which we estimate a granule surface area of $0.12\mu\text{m}^2$ (assuming spherical geometry).

Immunocytochemistry suggested that most EndoC- β H1 and - β H2 cells contained only insulin-labelled granules as but that a small subset of cells contained somatostatin- and/or glucagon-positive granules (Supplementary Fig. 6). Whereas insulin- and glucagon-positive cells were found only in EndoC- β H2, somatostatin-positive cells were found in both cell lines. Electron microscopy in conjunction with immunogold labeling indicated that somatostatin and insulin could be contained in the same granules (Fig. 7E). Transcript analysis confirmed high expression of insulin (*INS*) in both EndoC- β H1 and - β H2 cells and that glucagon (*GCG*) and somatostatin (*SST*) were expressed at particularly high levels in EndoC- β H2 cells. There was also high expression of the somatostatin receptors 1, 2 and 3 (*SSTR1-3*) (Supplementary Fig. 7 and Supplementary Table 1). Notably, addition of the SSTR2 antagonist CYN154806 doubled glucose-induced insulin secretion in EndoC- β H2 cells (Fig. 7F).

Discussion

We have characterized the electrophysiological properties of two human β -cell lines: EndoC- β H1 and - β H2. We confirmed that both cell lines are glucose-responsive with the stimulatory effect on insulin secretion limited to a 2- to 3-fold enhancement (8-10). However, higher stimulation was achieved in recent publication (16). We show that the stimulatory effect of glucose was associated with increases in $[Ca^{2+}]_i$ in a subset of the cells and stimulation of electrical activity. Here we discuss a few aspects of this work that we find particularly interesting and important.

K_{ATP} channel activity and action potential firing

The action potentials induced by glucose originated from a membrane potential of $\sim -50\text{mV}$ and usually peaked at $+5\text{mV}$ (or above). Both EndoC- βH1 and $-\beta\text{H2}$ are equipped with K_{ATP} channels. The measured K_{ATP} channel density at 1mM glucose in metabolically intact β -cells is 0.05nS/pF . As the cells had cell capacitances of $10\text{-}12\text{pF}$, these values correspond to a resting conductance of $\sim 0.5\text{nS}$ and an input resistance (the reciprocal of the resting conductance) as high as $2\text{G}\Omega$. The former value is only $\sim 10\%$ of the corresponding value in mouse β -cells but comparable to that observed in primary human β -cells (1). The high input resistance suggests that small currents (such as those resulting from the opening of individual ion channels) may produce a membrane depolarization large enough to trigger an action potential. This probably explains why EndoC- βH1 and $-\beta\text{H2}$ cells generate spontaneous action potentials and exhibit $[\text{Ca}^{2+}]_i$ transients at 1mM glucose.

Both increasing glucose (to 6 and 20mM) and application of the K_{ATP} channel inhibitor tolbutamide reduced the resting conductance by $50\text{-}60\%$. Thus, it appears that the net K_{ATP} density is $\sim 0.03\text{nS/pF}$ with the rest representing the contribution of other ion channels (or ‘leak’ around the recording electrode). The inhibitory effect of glucose could be reversed by diazoxide and the input conductance measured in the presence of this K_{ATP} channel activator was 3- to 6-fold that measured in the presence of glucose alone in EndoC- βH1 and $-\beta\text{H2}$ cells, respectively.

Voltage-gated ion channels

We found that the EndoC- βH1 and $-\beta\text{H2}$ cells express voltage-gated ion channel complements very similar to that found in primary human β -cells (23). Thus, action potential firing in these human β -cell lines involves activation of voltage-gated Na^+ ,

Ca^{2+} and K^{+} channels (28). Interestingly, most of EndoC- β H1 cells exhibit large-amplitude and long-duration action potentials. Using voltage-clamp command pulses based on recorded glucose-induced the action potentials, we found that this correlated with delayed and reduced activation of an iberiotoxin-sensitive K^{+} current (reflecting the opening of large-conductance Ca^{2+} -activated K^{+} channels; BK) and slower action potential repolarization. Voltage-clamp analyses of the current responses further suggest potential depolarization principally reflects opening of voltage-gated P/Q- and L-type Ca^{2+} channels with little contribution by voltage-gated Na^{+} channels. This is because action potentials originate from fairly depolarized membrane potentials (-55 to -50 mV). At these membrane potentials, the Na^{+} channels are largely inactivated, which explains why I_{Na} contributes only marginally to action potential firing at steady-state.

Correlating electrophysiology to gene expression

To establish how authentic EndoC- β H1 and - β H2 cells are as models of primary human β -cells, we correlated the electrophysiological data to gene expression. We found that there was, in general, good agreement at the transcript level between both cell lines and primary β -cells. Among the voltage-gated Na^{+} channels, *SCN8A* (Nav1.6) and *SCN9A* (Nav1.7) are expressed at particularly high levels. We detected high expression of L-type Ca^{2+} channel α -subunits *CACNA1C* (α 1C), *CACNA1D* (α 1D) and P/Q-type Ca^{2+} channels *CACNA1A*. In addition, very high expression of the T-type Ca^{2+} channel *CACNA1H* was detected. Expression of *CACNA1E* and *CACNA1B* (encoding R- and N-type Ca^{2+} -channels, respectively) were low in EndoC- β H1 and - β H2 cells.

Of the voltage-gated K^{+} channels, *KCNB2* (Kv2.2), *KCNQ2* (Kv7.2) and *KCNH2* (Kv11.1) are expressed at particularly high levels, mirroring what is seen in human β -

cells (29). High expression was also observed for the BK α -subunit gene *KCNMA1*.

However, despite the evidence for reduced BK activity in EndoC- β H1 cells compared to EndoC- β H2 cells, mRNA levels were comparable between the two cell lines.

Overall, the expression of these voltage-gated ion channels in EndoC- β H1 and - β H2 cells mirror that seen in primary human β -cells (29, 30).

Correlating ultrastructure, exocytosis and insulin secretion

Using immunofluorescence as well as electron microscopy, we show that both cell lines are well granulated, although the granules are not evenly distributed within the cell. The secretory granules are found at higher density in cytoplasmic projections (Supplemental Figs. 6-8). Granules are morphologically heterogeneous with a majority characterized by a very thin halo surrounding the electron dense core (insulin). The granule density (N_A) can be converted to the volume density (N_V) (Eq. 3, Supplementary material) and corresponded to ~ 13 and 20 granules/ μm^3 in EndoC- β H1 and β H2, respectively. When multiplied by the extranuclear cell volume, these values convert to $\sim 6,000$ and $15,000$ secretory granules/cell in EndoC- β H1 and - β H2 cells, respectively. This is not unlike the numbers found in primary β -cells (31, 32)

Exocytosis

The measurements of exocytosis also indicate that the late steps of insulin release largely recapitulate what is observed in primary β -cells. However, EndoC- β H1 cells could be distinguished from the - β H2 cells by the kinetics of secretion. Whereas most of exocytosis in the EndoC- β H2 cells occurred during the actual depolarization ('phasic exocytosis'), exocytosis in many EndoC- β H1 cells occurred after the depolarization

and when the membrane potential had returned to -70mV ('asynchronous exocytosis'). This suggests that the kinetics of exocytosis in EndoC- β H1 mirrors the depolarization-evoked $[Ca^{2+}]_i$ transients, which typically takes a few seconds to return to basal (33). This is consistent with the high expression of the high-affinity Ca^{2+} -sensor synaptotagmin 7 (*SYT7*) in EndoC- β H1 cells. However, this cannot be the sole explanation as equally high expression of *SYT7* is seen in EndoC- β H2 cells and yet exocytosis was 'phasic'.

Quantitatively, the exocytotic responses observed in EndoC- β H1 and - β H2 cells are impressive. A single 800ms depolarization produced a capacitance increase of 100 and 180fF in EndoC- β H1 and - β H2 cells, respectively. The granule area estimated from electron microscopy was $0.12\mu m^2$. With a specific capacitance of $10fF\mu m^{-2}$, this granule area predicts that each granule should add 1.2fF of capacitance upon fusion. Thus, the exocytotic responses equate to the discharge of 80-150 granules. This is ~1% of the total granule number. From the relationship between pulse duration and exocytosis, we estimate that a 10ms depolarization (the approximate duration of an action potential) will produce an exocytotic response of $\leq 1\%$ of that produce by the 800ms pulse (Fig. 5). Thus, we can estimate that each action potential will (on average) result in the release of 0.8-1.5 granules. Multiplying these values with the observed action potential frequency (1.45Hz) suggests that EndoC- β H1 and - β H2 cells release 100% and 50% of their total insulin content per hour! However, the $[Ca^{2+}]_i$ measurements suggests that only 20-25% of the cells respond to glucose. This implies that EndoC- β H1 and - β H2 release respectively about 20% and 10% of their insulin content per hour, in fair agreement with the secretion rates actually observed.

Impact of ‘polyhormonality’

At the mRNA level, glucagon and somatostatin levels equal that of insulin in EndoC- β H2. However, at the protein level only a relatively small fraction of cells are polyhormonal. Immunogold labeling in conjunction with electron microscopy revealed that these cells stored both insulin and somatostatin within the same granules. Interestingly, there was also relatively high expression of the somatostatin receptors 1, 2 and 3 (*SSTR1-3*), raising the possibility of autocrine inhibition. Indeed, when the cells were treated with 1 μ M CYN154806, glucose-induced insulin secretion was doubled. As this antagonist is selective for SSTR2, it is possible that even greater enhancement would have been observed if SSTR1 and SSTR3 had also been targeted. By contrast, glucagon receptors (*GCGR*) as well as GLP-1 receptor (*GLP1R*) are expressed at very low levels in both EndoC- β H1 and - β H2 cells. Thus, autocrine stimulation by GLP-1 or glucagon released from the EndoC- β H1 and - β H2 cells seems less likely.

Conclusion

We conclude that EndoC- β H1 and - β H2 cells are robust models of primary human β -cells. We acknowledge that the secretory response to glucose and tolbutamide appears relatively limited but work in other laboratories suggest that this can be improved and that the fold stimulation at high glucose can approach that seen in primary β -cells (16). We conclude that the EndoC- β H1 and - β H2 cells represent versatile models of primary human β -cells. They can be used for high-throughput pipelines (18) as well as detailed cell physiological studies of the insulin secretion defects associated with diabetes (19). Electrophysiologically, both EndoC- β H1 and - β H2 cells closely mirror primary human β -cells both at the functional and the gene expression levels. The fact that they are easy

to genetically modify makes them an attractive and powerful tool for future investigations of the relationship between gene expression and β -cell function.

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Authors contribution

BH, MG, AC, VN, AB, CB, IS, MC acquired data. BH, MG, AC, VN, MVDB, AT analyzed data. RS provided the cell lines. BH, MG, AC, ALG, PR designed experiments. BH and PR wrote the manuscript. BH is the guarantor of this work. The final version was read and approved by all authors.

Conflict of interest statement

The authors declare that no conflict of interest exists

List of abbreviations

GWAS: Genome-wide Association Study; GSIS: glucose-stimulated insulin secretion;
 SG: Secretory Granule; AUC: Area Under the Curve; AP: Action Potential; I: current
 (subscripts Na, Ca, K denote ions carrying the current); TTX: Tetrodotoxin; STX:
 Stromatoxin; IbTX: Iberiotoxin; TEA: Tetra-ethylammonium; INS: Insulin, SST:
 Somatostatin; GCG: Glucagon; N: nucleus, m: mitochondrion

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Legends to Figures

Figure 1 - Glucose-induced insulin secretion and $[Ca^{2+}]_i$ in EndoC- β H1 and - β H2

cells. (A) Basal insulin secretion elicited by 1mM glucose for 1 hour normalized to insulin content in EndoC- β H1 (black) and - β H2 (red). (B) Insulin secretion normalized to basal stimulated during 40min at 1, 6 or 20mM glucose and tolbutamide or diazoxide as indicated (n=3). *and # comparison to 1mM and 20mM glucose respectively, $P<0.05$, (ANOVA and Tukey). (C) Representative $[Ca^{2+}]_i$ responses (normalized to initial fluorescence; F/F_0) to 20mM glucose and 0.2mM tolbutamide, EndoC- β H1 (left) and - β H2 (right). Shown below are heatmaps from ≥ 120 cells from the same session (synchronized to representative traces) and displayed as F/F_0 (color scale at the bottom). (D) Quantification of the area under the curve (AUC) per minute for each condition (left, n=523 for 6mM and 225 cells for 20mM glucose over 4 experiments; right, n=442 for 6mM and 236 cells for 20mM glucose over 4 and 3 sessions for EndoC- β H1 and 2 respectively; * $p<0.05$, ANOVA and Tukey).

Figure 2 – K_{ATP} channel activity and action potential firing. (A) Representative

membrane potential recording made in EndoC- β H1 (top) and EndoC- β H2 (lower) at 1 and 20mM glucose (as indicated). (B) Action potential frequency in EndoC- β H1 and - β H2 cells at 1 and 20mM glucose. * $P<0.05$ (1mM glucose n=5 each, 20mM glucose n=3 and 4 for EndoC- β H1 and - β H2, respectively). (C) Examples of average action potentials (AP) recorded in two EndoC- β H1, and EndoC- β H2 cell and a primary human β -cell as indicated (average of at least 12 APs from the same recording). (D) Resting K_{ATP} channel activity in EndoC- β H1 and - β H2 cells measured at 1mM glucose (n=22 and 21 cells). (E) Effects of glucose, tolbutamide and diazoxide (added at the indicated concentrations) on whole-cell K_{ATP} channel activity. K_{ATP} channel activity has been

normalized to that at 1mM glucose. *and # $P < 0.05$ vs 1mM and vs 20mM glucose respectively (Student's t -test). Number of experiments (n) indicated below the figure. (F) Cell capacitance in EndoC- β H1 and - β H2 cells * $P < 0.05$ (n=30 and 32 cells).

Figure 3 – Voltage-gated Na^+ currents. (A) Voltage-gated Na^+ currents (I_{Na}) in EndoC- β H1 (left) and - β H2 cells (right) in the presence of Co^{2+} (1mM) with or without TTX (0.1 $\mu\text{g/ml}$; as indicated) during depolarizations from -70 to 0mV (representative of 24 and 11 cells). (B) Families of I_{Na} recorded during depolarizations from -70 (left) and -150mV (right) during depolarizations to indicated membrane potentials. (C) I_{Na} current-voltage relationships recorded from a holding potential of -70 or -150mV in EndoC- β H1 (upper; n=24) and - β H2 cells (lower; n=11). Current responses normalized to the peak current elicited by a depolarization from -70mV to 0mV. (D) I_{Na} activation when cells were held at -70mV in EndoC- β H1 and - β H2 cells. Curves were derived by fitting Eq. 1 (Supplementary Material) to current responses of individual experiments (n=26-23 cells). (E) Steady-state voltage-dependent inactivation of I_{Na} in EndoC- β H1 (red) and - β H2 cells (black) estimated from a two-pulse protocol: a standard test pulse to 0mV was preceded by 50ms conditioning pulse to membrane potentials between -150 and 0mV (n=26 and 32 cells). Data have been normalized to the maximum current during the test pulse ($h_{\infty} = I/I_{\text{max}}$). Responses have been fitted to Eq. 2 (Supplementary Material). (F) I_{Na} evoked by action potential-like voltage-clamp commands in EndoC- β H1 (black; n=26) and - β H2 cells (red; n=32).

Figure 4 – Voltage-gated Ca^{2+} currents. (A,C) Voltage-gated Ca^{2+} currents (I_{Ca}) evoked by membrane depolarizations between -70 and 0mV under control conditions and following sequential addition of ω -agatoxin (200nM), isradipine (10 μM) and

SNX482 (100nM) in EndoC- β H1 (A) and EndoC- β H2 (C). (B,D) Current voltage relationships normalized to maximum peak total current in each cell for the total I_{Ca} and the isolated P/Q (ω -agatoxin-sensitive), L- (isradipine-sensitive) and R-type (SNX482-sensitive) components in EndoC- β H1 (B) and EndoC- β H2 (D). (E-F) Current density (peak amplitude normalized to cell capacitance) histograms showing the maximum total as well as pharmacologically isolated R-, L- and P/Q-type Ca^{2+} current components in EndoC- β H1 (E) and EndoC- β H2 (F). (G) I_{Ca} evoked in EndoC- β H2 cells using a voltage-clamp command based on action potentials recorded in these cells. The traces shown correspond to the total I_{Ca} as well as the isolated P/Q-, L- and R-type components. Data are based on measurements in 5 EndoC- β H1 and 3 EndoC- β H2 cells.

Figure 5 – Voltage-gated K^+ currents. (A) Families of voltage-gated K^+ currents (I_K) in in EndoC- β H1 (*left*) and EndoC- β H2 (*right*) during voltage steps from -70mV to membrane potentials between -20 and +40mV. (B) Current density-voltage relationships in EndoC- β H1 (black) and EndoC- β H2 (red). Current density recorded in both cell lines at 0mV, inset. (C) I_K recorded during membrane depolarizations to 10mV under control conditions, after addition of stromatocin (Stx, 100nM) and iberiotoxin (Ibtx, 100nM) as indicated. Bottom panels show pharmacologically isolated K_V (Stx-sensitive) and BK Ibtx—sensitive) current components. (D) Voltage dependence of K_V and BK currents. Responses have been normalized to peak total I_K at +50mV. (E) I_K evoked in EndoC- β H1 and - β H2 cells using voltage-clamp commands based on the action potentials recorded in the respective cells under control conditions and the isolated Stx- and Ibtx-sensitive components. Data are based on measurements in 4 EndoC- β H1 and 3 EndoC- β H2 cells.

Figure 6 – Voltage dependence and kinetics of exocytosis. (A-B) Exocytosis evoked by 500ms depolarizations from -70mV to indicated membrane potentials (top) in EndoC- β H1 (black) and EndoC- β H2 cells (red). (B) Voltage dependence of exocytosis in EndoC- β H1 (black; n=6) and EndoC- β H2 cells (red; n=10). Responses are normalized to maximum response. (C-D) Exocytosis evoked by progressively longer (10-800ms) depolarizations from -70 to 0mV. (D) Relationship between pulse duration and exocytotic response in EndoC- β H1 (black; n=12) and EndoC- β H2 cells (red; n=14). Exocytosis is normalized to the response evoked by the 800ms depolarization (inset). (E-F) Representative trace of exocytosis evoked by trains of ten 500ms depolarizations from -70 to 0mV in EndoC- β H1 (black) and EndoC- β H2 cells (red). (F) Exocytotic response during the individual depolarizations in EndoC- β H1 (black, n=12) and EndoC- β H2 cells (red, n=14). Responses are normalized to the total increases in cell capacitance evoked by the trains in individual cells (inset).

Figure 7 – Ultrastructural analysis. (A) Electron micrograph of immunogold-labeled insulin (15nm gold particles) in EndoC- β H1 (left) and EndoC- β H2 cells (right). Abbreviations: SG, Secretory Granule; PM, Plasma Membrane; N, Nucleus. Scale bar: 1 μ m. (B-D) Quantification of the granule density (N_A , number of granule per cytoplasmic area; B), number of immunogold particles per SG (C) and granule cross-sectional area (D). Data in (A-D) representative for 10 cells of each type. (E) Some EndoC- β H2 cells have SG containing both insulin (10nm gold particles) and somatostatin (15nm gold particles). See also Supplementary Figure S6. Abbreviations: Ins, insulin; SST, somatostatin. Scale bar: 0.25 μ m. (F) Potentiation of insulin secretion at 20mM glucose by the SSTR antagonist CYN154806 (1 μ M). * P <0.05, t-test (n=3)

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