Diabetologia (2009) 52:1566–1578
DOI 10.1007/s00125-009-1382-z

ARTICLE

Somatostatin release, electrical activity, membrane currents and exocytosis in human pancreatic delta cells

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Received: 16 December 2008 / Accepted: 9 April 2009 / Published online: 14 May 2009

Abstract
Aims/hypothesis The aim of this study was to characterise electrical activity, ion channels, exocytosis and somatostatin release in human delta cells/pancreatic islets.
Methods Glucose-stimulated somatostatin release was measured from intact human islets. Membrane potential, currents and changes in membrane capacitance (reflecting exocytosis) were recorded from individual human delta cells identified by immunocytochemistry.
Results Somatostatin secretion from human islets was stimulated by glucose and tolbutamide and inhibited by diazoxide. Human delta cells generated bursting or sporadic electrical activity, which was enhanced by tolbutamide but unaffected by glucose. Delta cells contained a tolbutamide-insensitive, Ba^{2+}-sensitive inwardly rectifying K^+ current and two types of voltage-gated K^+ currents, sensitive to tetraethylammonium/stromatoxin (delayed rectifying, Kv2.1/2.2) and 4-aminopyridine (A current). Voltage-gated tetrodotoxin (TTX)-sensitive Na^+ currents contributed to the action potential upstroke but TTX had no effect on somatostatin release. Delta cells are equipped with Ca^{2+} channels blocked by isradipine (L), ω-agatoxin (P/Q) and NNC 55-0396 (T). Blockade of any of these channels interferes with delta cell electrical activity and abolishes glucose-stimulated somatostatin release. Capacitance measurements revealed a slow component of depolarisation-evoked exocytosis sensitive to ω-agatoxin.
Conclusions/interpretation Action potential firing in delta cells is modulated by ATP-sensitive K^+-channel activity. The membrane potential is stabilised by Ba^{2+}-sensitive inwardly rectifying K^+ channels. Voltage-gated L- and T-type Ca^{2+} channels are required for electrical activity, whereas Na^+ currents and P/Q-type Ca^{2+} channels contribute to (but are not necessary for) the upstroke of the action potential. Action potential repolarisation is mediated by A-type and Kv2.1/2.2 K^+ channels. Exocytosis is tightly linked to Ca^{2+}-influx via P/Q-type Ca^{2+} channels. Glucose stimulation of somatostatin secretion involves both K_{ATP} channel-dependent and -independent processes.

Keywords Electrophysiology · Exocytosis · Hormone secretion · Human islets · Islet physiology · Pancreatic delta cells · Patch-clamp · Somatostatin

Abbreviations

4-AP	4-Aminopyridine
$[Ca^{2+}]_i$	Intracellular Ca^{2+} concentration
K_{ATP} channel	ATP-sensitive K^+ channel
Kir	Inwardly rectifying K^+ channel
n_h	Slope factor
TEA	Tetraethylammonium
TTX	Tetrodotoxin
V_h	Voltage at which steady-state inactivation is half-maximal

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Electronic supplementary material The online version of this article (doi:10.1007/s00125-009-1382-z) contains supplementary material, which is available to authorised users.

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Introduction

Somatostatin-producing delta cells are the third most abundant cell type in pancreatic islets of Langerhans. Human islets contain about 10% delta cells, which is twice the proportion of mouse islets [1]. Pancreatic somatostatin has no major direct effect on glucose metabolism, but is a potent paracrine inhibitor of both insulin and glucagon secretion [2, 3]. Paracrine regulation of insulin and glucagon release is facilitated by the delta cells being distributed throughout the islet in man rather than confined to the islet periphery, as in rodents [4]. While in healthy adults somatostatin release is stimulated by glucose and tolbutamide, these effects are lost in type 1 and type 2 diabetes. This has been proposed to contribute to the impaired prandial suppression of glucagon secretion in diabetes [5, 6]. Activators of somatostatin receptors have therefore attracted interest as putative therapeutic agents [7].

There have only been a handful of studies investigating the mechanisms by which nutrients and pharmacological agents evoke somatostatin secretion from rodent delta cells [8–10] and even fewer data are available for human delta cells [11, 12]. Here we have explored the control of glucose-induced somatostatin release from isolated human islets and characterised the membrane currents involved in delta cell electrical activity and exocytosis.

Methods

Chemicals ω-Agatoxin IVA, ω-conotoxin GVIA, stromatoxin, tetrodotoxin (TTX) and SNX-482 were from Alomone (Jerusalem, Israel) or the Peptide Institute (Osaka, Japan). NNC 55-0396 and all other chemicals were purchased from Sigma-Aldrich (Gillingham, UK).

Islet isolation and cell culture Human islets were isolated in the Oxford Diabetes Research and Wellness Foundation Human Islet Isolation Facility from pancreases retrieved with appropriate ethical consent from non-diabetic heart-beating donors. For hormone secretion measurements, islets were cultured overnight in CMRL medium supplemented with 2 mmol/l L-glutamine. For electrophysiological experiments, freshly isolated islets were dispersed into single cells by trituration and plated onto plastic Petri dishes. The cells were cultured in RPMI medium containing 10 mmol/l glucose prior to the experiments.

Somatostatin secretion assay Batches of 10–20 islets (in triplicates) were pre-incubated in KRB containing 1 mmol/l glucose for 1 h, followed by a 1 h test incubation in KRB supplemented with glucose and inhibitors as indicated. The somatostatin content of the supernatant fraction was determined by RIA (Eurodiagnostica/IDS, Boldon, UK).

Electrophysiology Patch-clamp experiments were performed on isolated delta cells as previously described for human beta cells [13]. Detailed methods are provided in the Electronic supplementary material (ESM) Methods.

Immunocytochemistry Immunohistochemistry on human pancreatic tissue sections was performed as detailed in ESM Methods. Immunocytochemical identification of patch-clamped cells was performed as described previously [10, 13].

Quantitative RT-PCR Expression of ion channel isoforms was detected by quantitative RT-PCR on human islet total RNA (for details see ESM Methods).

Data analysis All data are expressed as means±SEM. Statistical significance was evaluated using Student's *t* test. All hormone secretion experiments were repeated using islets from at least three donors.

Results

Immunohistochemical localisation of delta cells in human pancreatic islets Figure 1a shows the intra-islet distribution of alpha, beta and delta cells in two human pancreatic islets (representative of 25 islets from three donors). The islets consist of clusters of beta cells surrounded by non-beta cells (mostly alpha cells). The somatostatin-secreting delta cells are scattered throughout the islet and often make direct contact with beta or alpha cells. These data are in agreement with previous reports [1, 4]. The number of delta cells is approximately the same in the head and the body of the pancreas [14].

Effects of glucose, diazoxide and tolbutamide on delta cell electrical activity and resting membrane conductance The identity of all delta cells used in the electrophysiological experiments was confirmed by immunocytochemical detection of somatostatin (Fig. 1b). The capacitance of delta cells averaged 4.3±0.1 pF (2.2–10.5 pF, n=91), which is close to the value obtained in mouse islets [9, 15]. Delta cells made up a significant proportion (about one-third) of the patch-clamped smaller (<6 pF) cells in most preparations.

Figure 2a shows a membrane potential recording from an electrically active delta cell exposed to 12 mmol/l glucose. The delta cell generated bursts of action potentials of variable duration from a baseline membrane potential of about −60 mV. Figure 2b shows a burst of action potentials

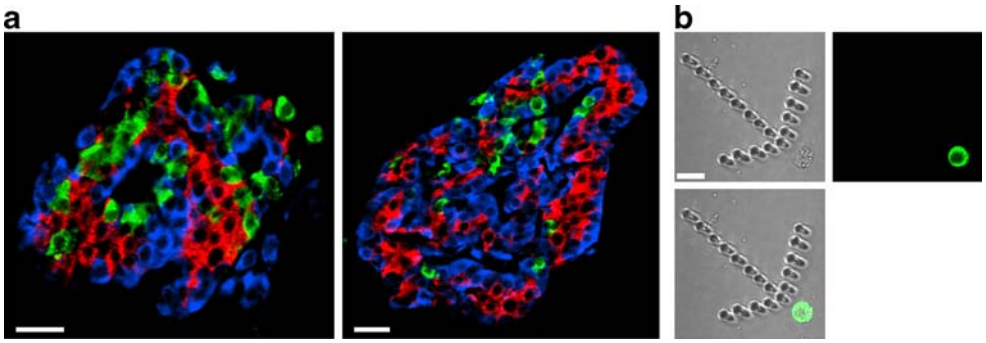

Fig. 1 Immunocytochemical detection of delta cells. **a** Human pancreatic tissue sections were stained with antibodies against insulin (red), glucagon (blue) and somatostatin (green). Two representative islets are shown (scale bars, 20 μm). **b** Identification of delta cells in dispersed islet preparations after patch-clamping by labelling with anti-somatostatin (green; scale bar, 10 μm)

on an expanded time base. The action potentials rose from a plateau of −40 mV and peaked at about −10 mV. Similar membrane potential oscillations were observed in three out of eight delta cells with spontaneous regenerative electrical activity. The remaining cells generated sporadic action potentials from a hyperpolarised membrane potential (cf. Fig. 5g, h). Many delta cells (seven out of 15) remained hyperpolarised despite the presence of glucose and tolbutamide. In these cells, action potential firing could be induced by injection of a depolarising current.

The ATP-sensitive K^+ channel (K_{ATP} channel) opener diazoxide reversibly repolarised the cell shown in Fig. 2a by 5 mV. This was associated with the suppression of electrical activity. Conversely, the K_{ATP} channel blocker tolbutamide prolonged the bursts (Fig. 2c). Varying the glucose concentration (between 1 and 20 mmol/l) did not affect electrical activity (data not shown).

The resting membrane conductance of delta cells averaged 0.5±0.1 nS at 6 mmol/l glucose (n=7). Changing the glucose concentration in the bath (between 1 and 20 mmol/l) had no consistent effect on the membrane conductance. Tolbutamide decreased the conductance in six out of 13 cells, with an average reduction by 35±3% (n=6; Fig. 2d). In five out of 15 cells tolbutamide depolarised the membrane or stimulated electrical activity. Diazoxide increased the membrane conductance in six out of nine cells, by an average of 179±88% (n=6).

We correlated these effects with changes in somatostatin release from intact human islets. At 1 mmol/l extracellular glucose, isolated human islets released 0.06±0.01% of their somatostatin content per hour (n=7 donors). Glucose increased somatostatin secretion concentration-dependently, with an average about 2.2- and 3.4-fold stimulation at 6 and 20 mmol/l, respectively (Fig. 2e; n=19 and 30 donors, respectively). The K_{ATP}-channel antagonist tolbutamide also enhanced somatostatin release at 1 mmol/l glucose but the effect was limited to about 1.8-fold stimulation (Fig. 2e). The K_{ATP}-channel opener diazoxide reduced the stimulatory effect of glucose (10 mmol/l) on somatostatin secretion by about 70% (Fig. 2e).

Presence of an inwardly rectifying K^+ current in delta cells Hyperpolarising voltage pulses from −70 mV elicited an inward current that underwent partial inactivation at negative potentials (Fig. 2f). This current was insensitive to tolbutamide and ZD7288, an antagonist of hyperpolarisation-activated cation channels (data not shown), but was blocked by a low concentration (100 μmol/l) of $BaCl_2$. As illustrated by Fig. 2g, the $BaCl_2$-sensitive current reversed at about −80 mV, close to the K^+-equilibrium potential expected under these experimental conditions (−89 mV), and exhibited strong inward rectification. Bath application of $BaCl_2$ destabilised the delta cell membrane potential but did not result in membrane depolarisation (Fig. 2h, n=4). These properties are compatible with 'classical' inwardly rectifying K^+ channels (Kir2.x). Quantitative PCR analysis of human islets confirmed expression of Kir2.2 and Kir2.3 (ESM Fig. 1a).

Voltage-gated K^+ currents Voltage-gated currents were studied in isolated delta cells using standard whole-cell recordings. Outward K^+ currents were observed during voltage-clamp depolarisations to −20 mV and above (Fig. 3a). The current amplitude increased nearly linearly with the voltage above −10 mV (Fig. 3b). The peak K^+ current after depolarisation to 0 mV averaged 483±90 pA, corresponding to a current density of 126±23 pA/pF (n=9). At more negative potentials (pulses to −20 to −10 mV), biphasic responses were observed that consisted of a rapidly activating transient and a slowly activating sustained component (Fig. 3a, inset).

The non-specific K^+-channel blocker tetraethyammonium (TEA) (10–20 mmol/l) reduced the peak outward current measured at 0 mV by 75±8% (p<0.01, n=5). After addition of TEA, a transient outward current was prominent also at positive potentials in eight out of 11 cells (Fig. 3c).

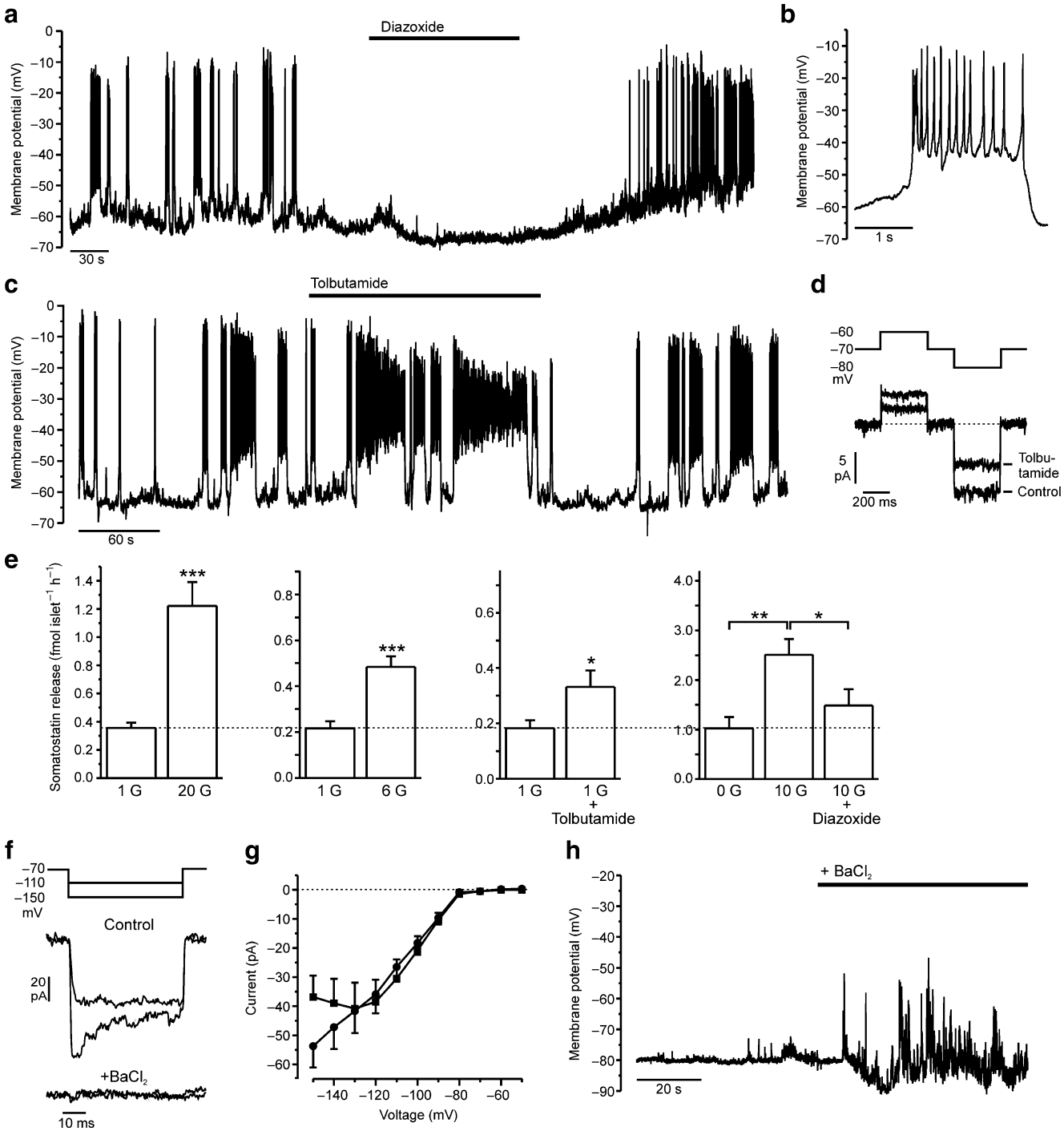

Fig. 2 Electrical activity and resting membrane conductance. **a** Effect of diazoxide (100 μmol/l) on delta cell membrane potential in the presence of 12 mmol/l glucose. **b** A burst of action potentials on an expanded time base. **c** Effects of tolbutamide (100 μmol/l) on electrical activity (at 20 mmol/l glucose; same cell as in **a**). **d** Resting membrane conductance before and after application of tolbutamide (at 20 mmol/l glucose). **e** Effects of 20 mmol/l glucose (20 G), 6 mmol/l glucose (6 G), tolbutamide (100 μmol/l, at 1 mmol/l glucose) and diazoxide (100 μmol/l, at 10 mmol/l glucose) on somatostatin secretion from isolated human islets. Data are means±SEM from 30 (20 mmol/l glucose), 19 (6 mmol/l glucose), four (tolbutamide) and five (diazoxide) preparations of human islets, respectively. The graphs have been adjusted to the same bar height for basal secretion (1 or 0 mmol/l glucose). *$p<0.05$, **$p<0.01$, ***$p<0.001$. **f** $BaCl_2$-sensitive (100 μmol/l) inward current activated by hyperpolarising voltage pulses from resting potential. The traces are shown after subtraction of leak currents using a P/4 protocol. **g** Current–voltage relationship of the $BaCl_2$-sensitive conductance for peak (circles) and sustained (squares) current ($n=7$). Recordings in **f** and **g** were performed in the presence of tolbutamide (100 μmol/l). **h** Effect of $BaCl_2$ (100 μmol/l) on membrane potential (in the presence of 100 μmol/l tolbutamide)

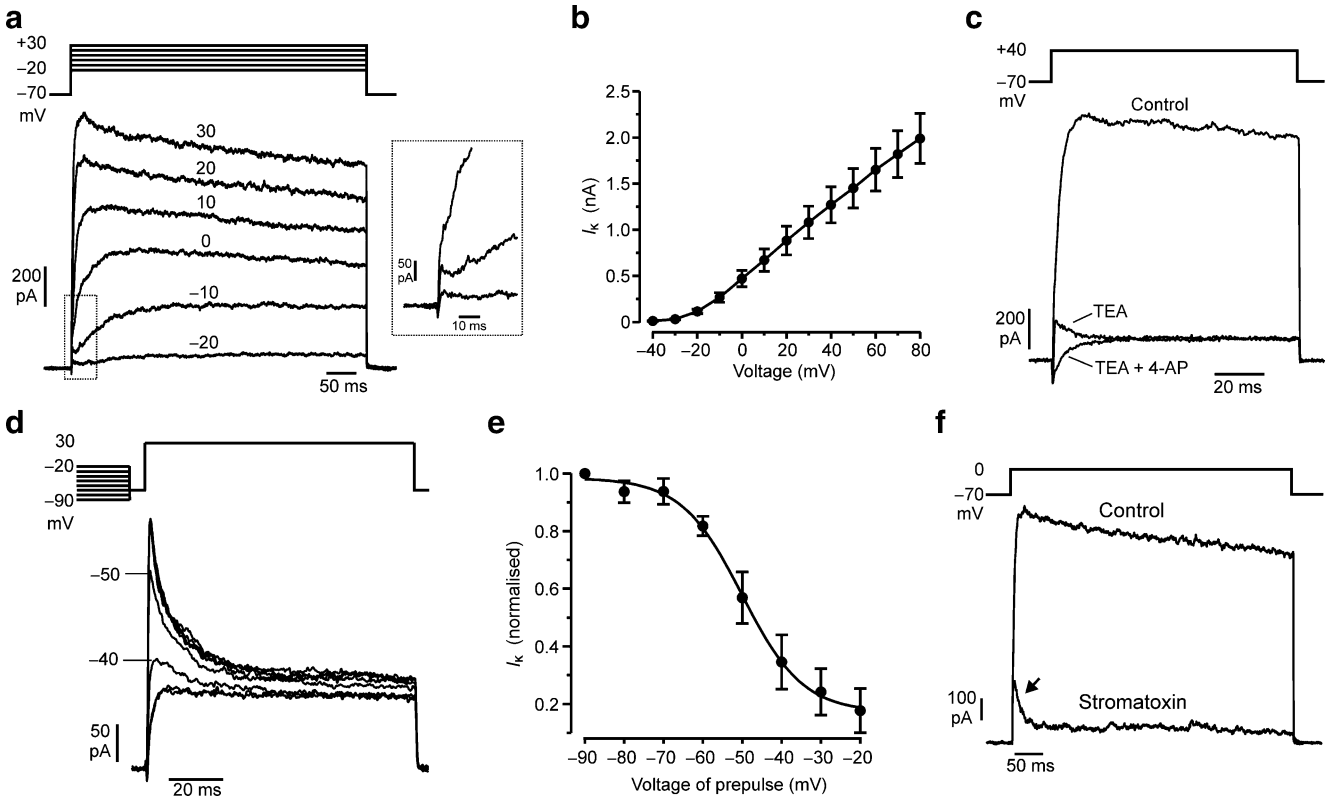

Fig. 3 Voltage-gated K^+ currents. **a** Outward K^+ currents elicited by 500 ms voltage-clamp depolarisations to the indicated potentials. The inset shows the initial part of the responses at −20, −10 and 0 mV as indicated by the box. **b** Current–voltage relationship for outward K^+ currents (n=8, means±SEM). **c** K^+ currents triggered by 100 ms depolarisations under control conditions, in the presence of TEA (10 mmol/l) alone and in the presence of TEA and 4-AP (5 mmol/l). **d** Transient K^+ current (in the presence of 10 mmol/l TEA) elicited by depolarisations to +30 mV, preceded by 200 ms conditioning pulses to potentials between −90 and −20 mV. The responses obtained after conditioning pulses to −50 and −40 mV are indicated. **e** Voltage-dependent inactivation of the transient K^+ current, measured as described in **d** (n=4). The currents have been normalised to the response after the conditioning prepulse to —100 mV. Means±SEM. A Boltzmann fit of the data is superimposed. **f** K^+ currents before (control) and after application of stromatoxin (100 nmol/l). Note the stromatoxin-resistant transient current (arrow)

The transient current had an average peak amplitude of 130±21 pA at 0 mV (n=8). Assuming m^4h kinetics [16], the time constants of activation and inactivation τ_m and τ_h averaged 0.42±0.09 ms and 10.1±0.7 ms, respectively (at +10 mV, n=7). The TEA-resistant transient current was sensitive to 4-aminopyridine (4-AP; 5 mmol/l, Fig. 3c). These properties are characteristic of A-type K^+ currents. At very positive voltages, the A current was often visible even when K^+ was replaced by Cs^+ in the intracellular solution (ESM Fig. 2a).

Voltage-dependent inactivation of the A current was investigated using a two-pulse protocol, consisting of a conditioning prepulse to increasingly positive voltages followed by an activating test pulse to +30 mV (Fig. 3d). A Boltzmann fit to the data yielded values of −51±1 mV for half-maximal inactivation (V_h) and 6.6±1.7 mV for the slope factor (n_h; n=4; Fig. 3e).

To elucidate the molecular identity of the channel(s) mediating the slowly activating, sustained and TEA-sensitive outward current (=delayed rectifier current), we applied the Kv2.1/2.2-specific antagonist stromatoxin. Expression of Kv2.2 and Kv2.1 channels in human islets has been reported previously [13, 17]. Stromatoxin reduced the peak and sustained outward currents during 500 ms depolarisations to 0 mV by 62±10% and 96±2%, respectively (p<0.05, n=4), without affecting the A current (Fig. 3f; arrow).

Stromatoxin (100 nmol/l) was without effect on glucose-stimulated somatostatin secretion (Fig. 4a). TEA (10 mmol/l) significantly increased basal (1 mmol/l glucose) somatostatin release, but was ineffective at stimulatory glucose concentrations (Fig. 4b). The stimulation at low glucose we partly attribute to inhibition of K_{ATP} channels [18]. The A-type K^+ current blocker 4-AP (5 mmol/l) had no effect on somatostatin secretion from islets at basal glucose levels (1 mmol/l; data not shown) but paradoxically abolished the stimulatory effect of 20 mmol/l glucose (Fig. 4c). Similar results were obtained for all antagonists when secretion was stimulated by 6 mmol/l glucose (ESM Table 1).

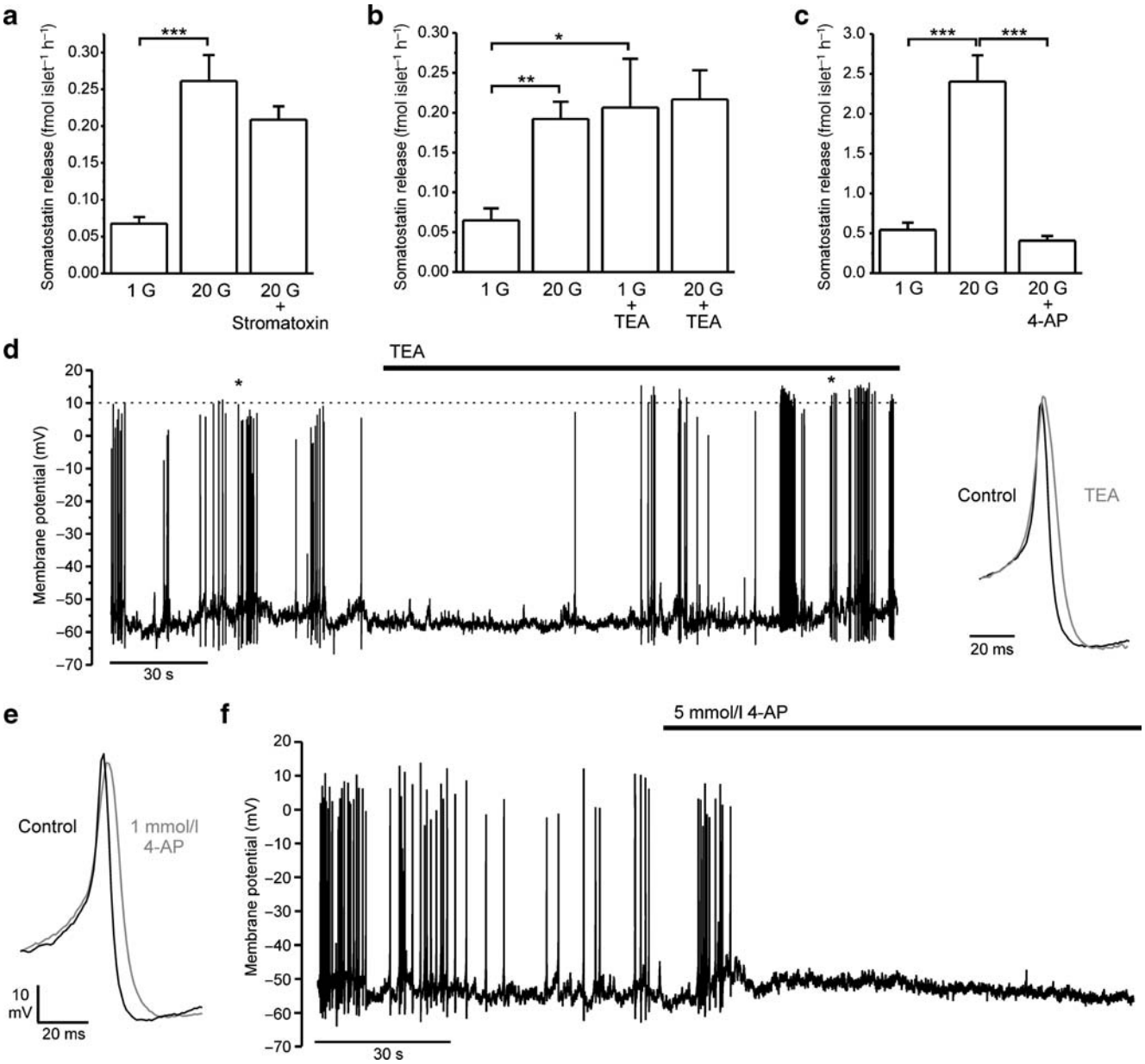

Fig. 4 Effects of blockers of voltage-gated K^+ channels on somatostatin release and electrical activity. **a–c** Somatostatin release from islets at 1 or 20 mmol/l glucose (1 G, 20 G) in the absence and presence of stromatoxin (100 nmol/l; **a**), TEA (10 mmol/l; **b**) or 4-AP (5 mmol/l; **c**) as indicated. Means±SEM (*$p<0.05$, **$p<0.01$, ***$p<0.001$, $n=9$–12). **d** Effect of TEA (10 mmol/l) on glucose (10 mmol/l)+ tolbutamide (0.1 mmol/l)-induced electrical activity. Action potentials marked by asterisks are shown on an expanded time base (right panel). **e** Single action potentials recorded in the absence (control) and presence of 1 mmol/l 4-AP. **f** Effect of 4-AP (5 mmol/l) on electrical activity recorded in the presence of 10 mmol/l glucose and 0.1 mmol/l tolbutamide. Records in **d–f** were obtained from the same delta cells after injection of 9 pA of current to evoke action potential firing

Inhibition of delayed-rectifier K^+ currents using TEA broadened delta cell action potentials (+72±11%) and increased their peak voltage (from 4±3 to 14±2 mV; Fig. 4d; $n=3$). Blocking about 50% of A currents using a low concentration (1 mmol/l) of 4-AP also increased action potential duration (Fig. 4e) or amplitude but the effects were weaker than those of TEA ($n=3$). Complete block of A currents by 5 mmol/l 4-AP abolished glucose- or tolbutamide-induced action potential firing in all cells tested (Fig. 4f; $n=5$). This inhibition was not associated with any consistent change of the membrane potential.

Voltage-gated Na^+ currents Voltage-gated inward currents were studied after replacing K^+ by Cs^+ in the pipette solution and including TEA in the bath solution. Under these conditions, voltage-clamp depolarisations to 0 mV

elicited an inward current consisting of transient and sustained components. The latter was abolished by 1 mmol/l Co^{2+}, a broad-spectrum blocker of voltage-gated Ca^{2+} channels (n=7), whereas the former was sensitive to the Na^+-channel blocker TTX (0.1 μg/ml, n=4; Fig. 5a).

The TTX-sensitive Na^+ current was activated by depolarisation to −30 mV and above and peaked at 0 mV (Fig. 5b). Voltage-dependent inactivation of Na^+ currents was studied using a two-pulse protocol. Na^+ currents inactivated significantly after prepulses to −50 mV and above (Fig. 5c); V_h and n_h averaged −45±3 mV and 7±1 mV (n=7), respectively (Fig. 5d). Close inspection revealed that about 10% of the current inactivated at more negative voltages (above −80 mV). Thus, the inactivation curve can be described as the sum of the components with V_h values of −75 mV (about 10%) and −45 mV (about 90%).

When applied to electrically active delta cells, TTX reduced the action potential amplitude by about −10 mV (Fig. 5g, n=3). Despite this, TTX did not inhibit somatostatin secretion evoked by 6 or 20 mmol/l glucose (Fig. 5e, f).

Voltage-gated Ca^{2+} currents Voltage-gated Ca^{2+} currents were studied after blocking Na^+ currents with TTX. Ca^{2+} currents were observed after depolarisations to −50 mV and above (Fig. 6a). Assuming m^2h kinetics, the time constant of activation (τ_m) during a pulse to 0 mV averaged 0.32± 0.03 ms (n=6). Ca^{2+} current inactivation at zero mV was usually biphasic, with time constants of 5.1±0.9 ms and 68±14 ms for the fast and slowly inactivating component, respectively (n=6).

The Ca^{2+} channels contributing to the voltage-gated Ca^{2+} current were characterised using specific antagonists. The L-type Ca^{2+}-channel blocker isradipine (10 μmol/l) predominantly reduced the transient component (Fig. 6b). On average, the integrated Ca^{2+} current evoked by 100 ms depolarisations to 0 mV was decreased by 15±4% (p<0.01, n=10). The P/Q-type-specific antagonist ω-agatoxin IVA strongly inhibited the sustained Ca^{2+} current (Fig. 6b) and decreased the average charge entry by 66±7% (p<0.01, n=5). SNX482 and ω-conotoxin GVIA, blockers of R- and N-type Ca^{2+} channels respectively, had no consistent effects on the Ca^{2+} currents in human delta cells (n=2 and n=4, respectively).

Figure 6c shows the voltage dependence of the isradipine- and ω-agatoxin-sensitive Ca^{2+} current components (data presented as integrated current normalised to cell capacitance). Activation of both the total and the L-type (isradipine-sensitive) current was biphasic, with a plateau between −40 and −20 mV. The P/Q-type (ω-agatoxin-sensitive) current was negligible at voltages below −20 mV and maximal at 0 mV.

Figure 6d shows the inward Ca^{2+} current elicited by a voltage ramp between −100 and +40 mV (after blockade of L-type channels). This stimulation paradigm revealed a low-voltage activated current activating above −60 mV and reaching a maximum at −40 mV that was specifically blocked by the T-type Ca^{2+}-channel antagonist NNC 55-0396; the secondary peak at membrane potentials about zero can be attributed to the high-voltage activated P/Q-type current. The low-voltage activated current was observed in 11 out of 12 delta cells tested. Depolarisation to −30 mV (to selectively activate the T-type Ca^{2+} current) in these cells elicited a transient Ca^{2+} current (Fig. 6e). Voltage-dependent inactivation of T-type currents was investigated using a two-pulse protocol (Fig. 6e); V_h and n_h averaged −66±3 mV and 6±2 mV, respectively (n=7). It is likely that during pulses to 0 mV (cf. Fig. 6b) the T-type current was obscured by simultaneous activation of Cs^+-mediated A-type outward currents (see ESM Fig. 2a), thus accounting for the apparent absence of an inactivating Ca^{2+} current component in the simultaneous presence of ω-agatoxin and isradipine.

We investigated the effect of Ca^{2+}-channel blockade on electrical activity in delta cells. Figure 7a shows a cell with slow bursting electrical activity where application of the L-type antagonist isradipine completely blocked action potential firing while not preventing the underlying slow waves. Isradipine also blocked action potentials evoked by current injection (n=2, data not shown). The P/Q-type channel ω-agatoxin IVA caused a small reduction of action potential amplitudes (about 5 mV, Fig. 7b, n=2). Subsequent addition of the T-type channel antagonist NNC 55-0396 strongly suppressed action potential firing (Fig. 7b, n=2).

Next the effects of subtype-specific Ca^{2+}-channel antagonists on somatostatin release were characterised. As seen in Fig. 7c–e, the L-type blocker isradipine, the P/Q-type antagonist ω-agatoxin IVA and the T-type specific blocker NNC 55-0396 all strongly suppressed glucose-stimulated somatostatin release. None of the compounds significantly influenced somatostatin release at low (1 mmol/l) glucose (not shown). Similar inhibitory effects on glucose-induced somatostatin secretion were obtained at 6 mmol/l glucose (ESM Table 1).

Slow exocytosis in human delta cells The exocytotic properties of human delta cells were characterised by capacitance measurements. Exocytosis was stimulated by voltage-clamp depolarisations to 0 mV of increasing duration, triggering Ca^{2+} influx through voltage-gated Ca^{2+} channels. Whereas exocytosis was usually negligible during short (≤50 ms) depolarisation pulses, longer pulses elicited progressively larger responses (Fig. 8a). A continuing increase in cell capacitance was observed after the end of the depolarisation ('slow component' of exocytosis). In some cells the slow component showed a delayed acceleration after the end of the depolarising pulse (Fig. 8b).

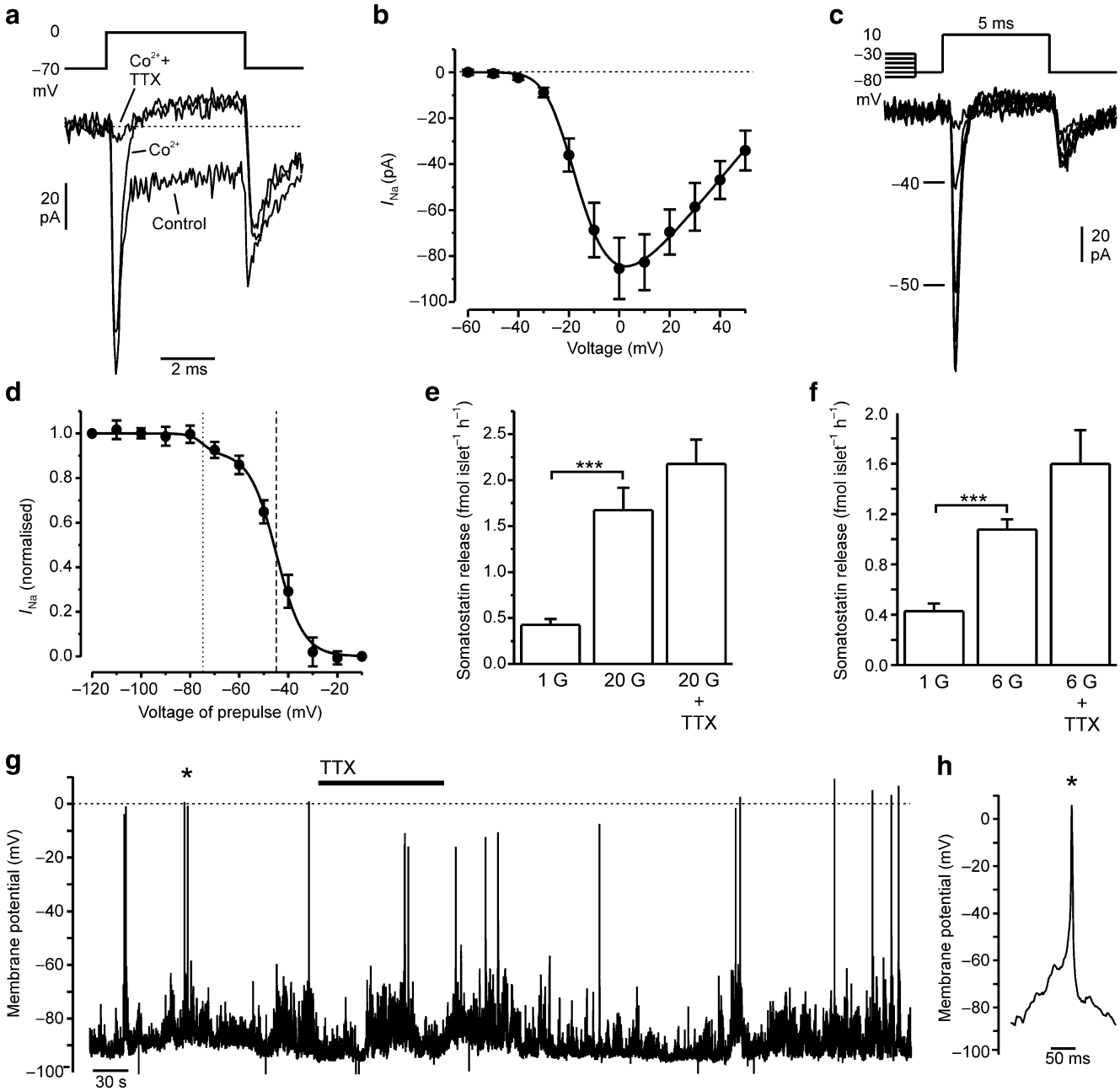

Fig. 5 Voltage-gated Na^+ currents. **a** Inward currents elicited by depolarisation to 0 mV (with Cs^+-containing pipette solution) under control conditions, after addition of $CoCl_2$ (1 mmol/l) and in the combined presence of $CoCl_2$ and TTX (0.1 μg/ml). **b** Current–voltage relationship of voltage-gated Na^+ currents (recorded in the presence of 1 mmol/l $CoCl_2$; n=7). A current–voltage fit has been superimposed. Means±SEM. **c** Na^+ currents (in the presence of 1 mmol/l $CoCl_2$) elicited by a depolarisation pulse to 10 mV preceded by a 50 ms conditioning pulse to potentials between −80 and −30 mV. The responses after conditioning pulses to −50 and −40 mV are indicated. **d** Relationship between voltage of the conditioning pulse and Na^+ current amplitude, obtained using the protocol described in **c** (n=7, means±SEM). The currents have been normalised to the responses following a prepulse to −120 mV. A double Boltzmann fit of the data is superimposed. The dotted and dashed vertical lines indicate the V_h of two components accounting for about 8% and 92%, respectively, of the total Na^+ current. **e**, **f** Somatostatin secretion at 1, 6 and 20 mmol/l glucose (1/6/20 G) in the absence and presence of TTX (0.1 μg/ml; ***p<0.001, n=9, means±SEM). **g** Effect of TTX (0.1 μg/ml) on electrical activity recorded in the presence of 6 mmol/l glucose and 0.1 mmol/l tolbutamide. **h** An action potential (marked by the asterisk in **g**) on an expanded time scale

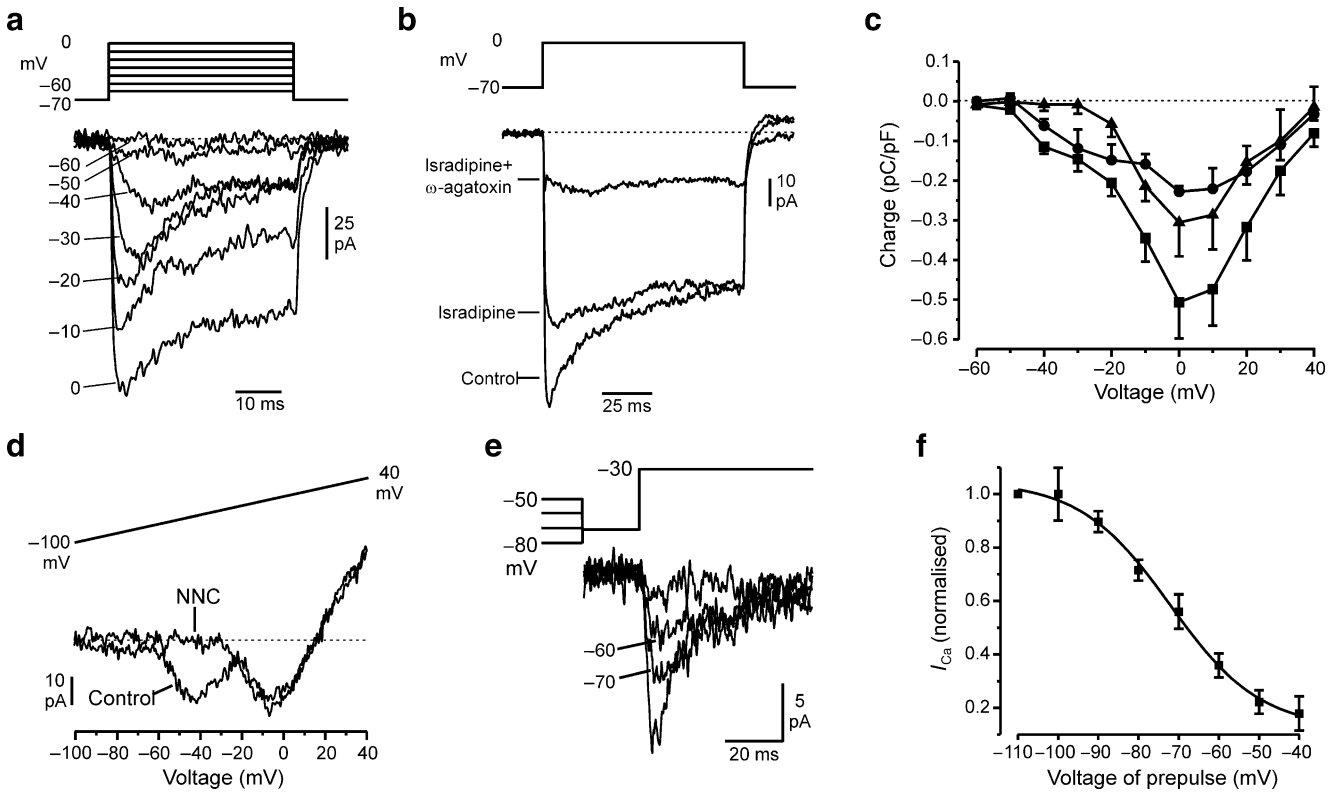

Fig. 6 Voltage-gated Ca^{2+} currents. **a** Ca^{2+} currents (in the presence of 0.1 μg/ml TTX) elicited by voltage-clamp depolarisations to the indicated potentials. **b** Ca^{2+} currents evoked by depolarisation to 0 mV under control conditions, after application of isradipine (10 μmol/l) and in the combined presence of isradipine and ω-agatoxin IVA (200 nmol/l). **c** Relationship between voltage of depolarisation pulse (40 ms duration) and integrated Ca^{2+} current (normalised to cell capacitance) for total (squares, n=7), L-type (circles, n=4) and P/Q-type (triangles, n=4) currents. Means±SEM. L- and P/Q-type currents were reconstructed by subtracting the responses after addition of isradipine and ω-agatoxin IVA, respectively, from those obtained before addition of the blockers. **d** Currents measured during a 100 ms voltage-ramp from −100 to +40 mV (in the presence of 10 μmol/l isradipine) before (control) and after addition of NNC 55-0396 (3 μmol/l). **e** T-type Ca^{2+} currents elicited by depolarisation pulses to −30 mV preceded by 500 ms conditioning pulses to voltages between −90 and −50 mV. The responses following conditioning pulses to −70 and −60 mV are indicated. The recording was made in the presence of 10 μmol/l isradipine to eliminate contribution of low-voltage activated L-type currents (see **c**). **f** T-type Ca^{2+} current plotted against the voltage of the conditioning pulse (data were obtained using the protocol described under **e**, n=7, means±SEM). Currents have been normalised to the responses at −110 mV. A Boltzmann fit is superimposed

Figure 8c compares responses from a human delta cell and a human beta cell. While the total capacitance increase was similar in both cells, the slow component was substantially larger and displayed slower kinetics in the delta cell. On average, the slow component accounted for 54±9% and 55±6% (n=11) of the total increase in delta cell membrane capacitance elicited by 200 and 500 ms depolarisations, respectively (Fig. 8d). For comparison, in human beta cells the slow component is responsible for 15±4% and 22±3% of the responses to 200 ms and 500 ms depolarisations (n=16). In human delta cells, exocytosis elicited by a 500 ms depolarisation to zero mV continued beyond the pulse with a time constant of 0.34±0.02 s (n=8). The corresponding value in mouse delta cells is 0.12±0.03 s (n=5).

Exocytotic responses were small after depolarisations to −20 mV and below but increased steeply during pulses to more positive potentials with a peak at 0 mV (Fig. 8e). This voltage-dependence closely resembles that of the P/Q-type Ca^{2+} current (cf. Fig. 6c). The P/Q-type Ca^{2+}-channel blocker ω-agatoxin IVA almost fully suppressed the exocytotic response (Fig. 8f); exocytosis elicited by 500 ms depolarisation was reduced by 95±2% (n=4).

Discussion

Delta cells are extremely rare in dispersed mouse islet preparations [15] and, possibly because of their elaborate morphology, difficult to isolate by cell-sorting techniques [19]. Consequently, there have been few studies investigating the cellular control of delta cell somatostatin secretion. Recently, mouse delta cells were studied in intact mouse islets but this technique is technically challenging. Here we

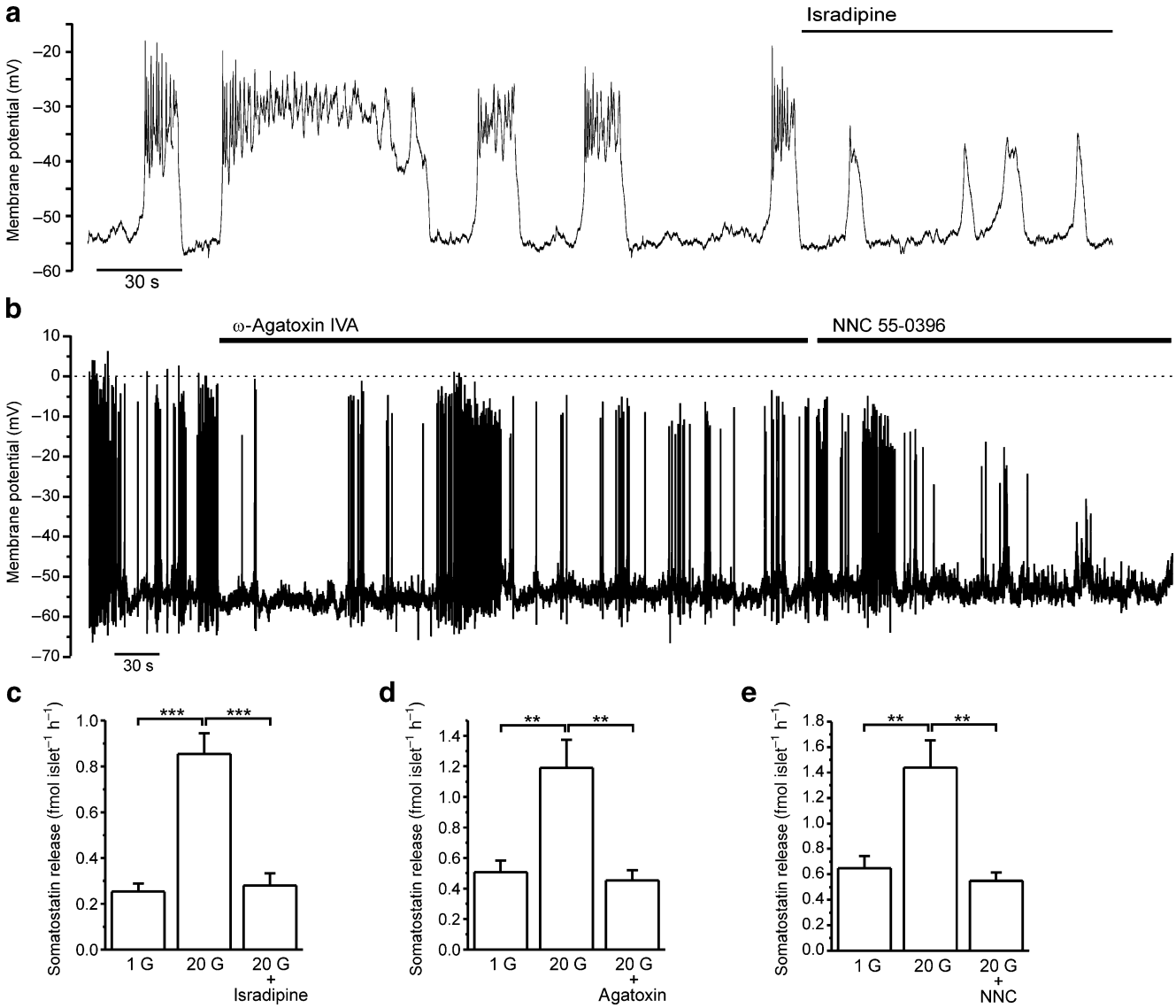

Fig. 7 Effects of Ca^{2+} channel blockers on electrical activity and glucose-stimulated somatostatin release. **a** Membrane potential recording in the presence of 10 mmol/l glucose and 0.1 mmol/l tolbutamide. Isradipine (10 μmol/l) was added as indicated by the bar. **b** Effects of ω-agatoxin IVA (200 nmol/l) and NNC 55-0396 (3 μmol/l) on electrical activity recorded in the presence of 10 mmol/l glucose and 0.1 mmol/l tolbutamide with injection of 9 pA depolarising current. **c–e** Somatostatin secretion at 1 and 20 mmol/l glucose (1/20 G) in the absence and presence of isradipine (10 μmol/l; **c**), ω-agatoxin IVA (200 nmol/l; **d**) or NNC 55-0396 (1 μmol/l: **e**) as indicated (**$p<0.01$, ***$p<0.001$, $n=9–12$, means±SEM)

have performed the first characterisation of ion channels, electrical activity and exocytosis in human delta cells. The ion channel complements of human and mouse delta cells are compared in ESM Table 2.

Some human delta cells generate bursting electrical activity similar to that which has previously been observed in mouse (but not human) beta cells [13, 20]. These bursts of action potentials are likely to underlie the intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ oscillations reported in delta cells [11, 12, 21, 22], although the frequency of the $[Ca^{2+}]_i$ oscillations was slightly lower (<1/min) than that of the electrical bursts observed here (2–5/min). It has been proposed, based on both experimental and theoretical considerations, that oscillatory electrical activity in mouse beta cells depends on cell coupling [23, 24]. However, the finding that high-frequency bursts of action potentials can be recorded from individual human delta cells suggests that cell coupling is not required for such electrical activity to occur. The ion channels responsible for the membrane potential oscillations in delta cells are unclear. In mouse beta cells, K_{ATP} channels and low-conductance Ca^{2+}-activated K^+ channels are thought to be important for the bursting pattern [25–27]. The K_{ATP}-channel antagonist tolbutamide stimulated somatostatin secretion from isolated

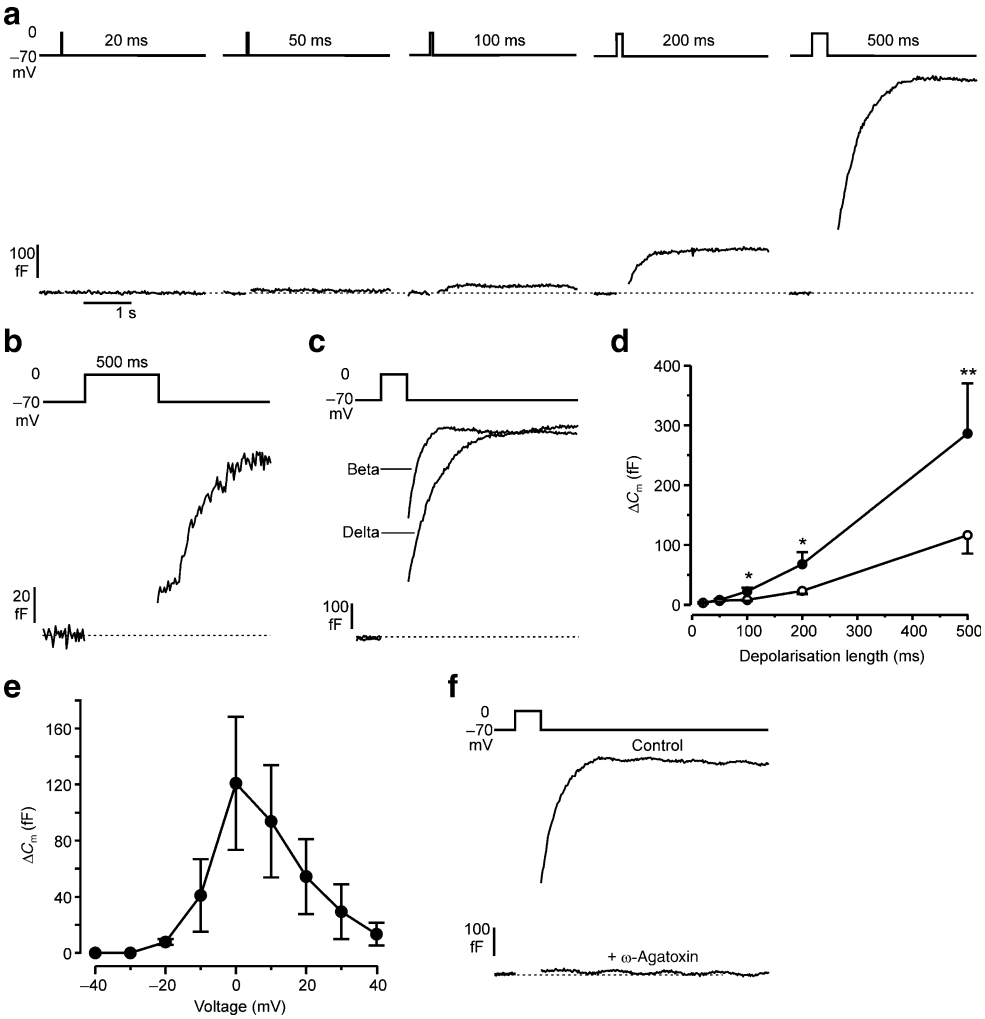

Fig. 8 Depolarisation-evoked exocytosis. **a** Capacitance increase triggered by voltage-clamp depolarisations to 0 mV of the indicated duration. **b** Capacitance response to a 500 ms depolarisation on an expanded time scale. Note two kinetic components of the post-depolarisation increase. **c** Comparison of capacitance responses to 500 ms depolarisations obtained in a human beta cell and a human delta cell. **d** Capacitance responses (ΔC_m) to depolarisations of the indicated duration, including (black circles) and excluding (white circles) the continued increase after the end of the depolarisation pulse ('slow component'; *$p<0.05$, **$p<0.01$; $n=11$, means±SEM). **e** Relationship between voltage of 500 ms depolarisation pulses and capacitance increase (ΔC_m; $n=5$). Means±SEM. **f** Capacitance increase elicited by 500 ms depolarisations to 0 mV before and after application of 200 nmol/l ω-agatoxin IVA

human islets and electrical activity in delta cells, while the K_{ATP}-channel opener diazoxide had inhibitory effects. This adds to previous evidence that K_{ATP} channels are expressed in rodent and human delta cells and are important for the regulation of electrical activity, cytoplasmic Ca^{2+} signals and somatostatin release [6, 8–11, 21, 28]. The observation that tolbutamide prolongs both the $[Ca^{2+}]_i$ oscillations [11] and the periods of electrical activity (Fig. 1c) suggest that K_{ATP} channels are also involved in the generation of bursts of action potentials in human delta cells.

We also identified a second background K^+-conductance in delta cells with properties closely resembling 'classical' inwardly rectifying K^+ channels. This current has previously not been described in pancreatic islet cells. PCR analysis of human islets revealed expression of *KCNJ12* (Kir2.2; 90% of the transcripts) and *KCNJ4* (Kir2.3; 10% of the transcripts) and it is tempting to attribute the observed inwardly rectifying K^+ current to these channels. In cardiomyocytes, inwardly rectifying K^+ channels stabilise the resting membrane potential and maintain the plateau phase of the prolonged action potentials [29, 30]. The finding that blockade of the inwardly rectifying K^+ current by Ba^{2+} resulted in the emergence of chaotic membrane potential oscillations suggests a role for inward rectifiers in membrane potential stabilisation in delta cells.

Human delta cells possess two types of voltage-gated K^+ currents. The first type activates and inactivates rapidly, undergoes voltage-dependent inactivation, is resistant to TEA and blocked by 4-AP, thus displaying all characteristics of A currents. The second type of K^+ current is of the delayed-rectifier type and sensitive to TEA and the Kv2.1/2.2 specific antagonist stromatoxin. This confirms a previous study reporting expression of Kv2.2 in human delta cells [17]. Inhibition of the sustained outward K^+ current using TEA increased action potential duration and amplitude and stimulated/tended to stimulate somatostatin secretion at 1 and 6 mmol/l glucose (Fig. 4 and ESM Table 1). Blocking the A current using a low (1 mmol/l) concentration of 4-AP likewise affected action potential duration or height. However, when tested at a maximally inhibitory concentration, 4-AP paradoxically suppressed delta cell electrical activity. The latter effect echoes the inhibition of glucose-stimulated somatostatin release. Glucose-induced insulin secretion (human

beta cells lack A currents [13]) from the same islets was unaffected by 4-AP (ESM Fig. 2b), making it unlikely that the inhibition of somatostatin secretion reflects an unspecific action. It is possible that the A current plays a role in the after-hyperpolarisation of the action potential and that this is required for the maintenance of delta cell electrical activity; perhaps by allowing recovery from voltage-dependent inactivation of other conductances involved in action potential firing. Clearly, the precise role of the A current in the control of delta cell electrical activity and somatostatin secretion merits further investigation.

Like mouse delta cells, human delta cells are equipped with TTX-sensitive, voltage-gated Na^+ channels, albeit with a slightly shifted voltage-dependence of inactivation (V_h at −45 mV compared with −28 mV in mice [9]). Although these channels contribute to the upstroke of the delta cell action potential (Fig. 5f), inhibition of their activity using TTX did not affect somatostatin secretion (Fig. 5e). This may reflect the relatively small amplitude of the Na^+ current. The inactivation of the Na^+ current was biphasic. Thus, 10% of the current appeared to inactivate fully at voltages more negative than −60 mV, whereas the inactivation of the remaining 90% occurred at 20–30 mV more positive voltages. It is possible that this behaviour reflects the existence of distinct Na^+ channel isoforms. Human islets produce high levels of both Na^+ channel, voltage gated, type VIII, alpha subunit (SCN8A) and Na^+ channel, voltage-gated, type IX, alpha subunit (SCN9A) α-subunits, and of Na^+ channel, voltage-gated, type I, beta (SCN1B) and Na^+ channel, voltage-gated, type III, beta (SCN3B) β-subunits [13].

Like human beta cells and mouse delta cells, human delta cells express multiple types of voltage-gated Ca^{2+} currents. Interestingly, all of these appear to be essential for glucose-induced somatostatin secretion. L-type channels are, as in human beta cells [13], indispensable for action potential generation (Fig. 7a). This may reflect the fact that L-type Ca^{2+} channels activated at voltages as negative as −40 mV (Fig. 6c). The current–voltage relationship of L-type channels was biphasic, compatible with expression of both CaV1.3 (activating at more negative potentials) and CaV1.2 (activating at higher voltages) [13, 31]. Similar to human beta cells [13], low-voltage activated T-type channels are also important for action potential initiation and upstroke (Fig. 7b). P/Q-type channels, the dominant (66%) high-voltage activated Ca^{2+} channel type in human delta cells, are presumably activated downstream of T- and L-type channels and are closely coupled to the exocytosis of secretory granules (Fig. 8f).

Glucose (both 6 and 20 mmol/l) evoked a robust stimulation of somatostatin secretion from isolated human islets. This is in line with in vivo studies demonstrating an increase in plasma somatostatin levels upon glucose intake in healthy individuals [5]. Surprisingly, glucose had no consistent effects on the delta cell membrane potential and electrical activity. This is in agreement with the variable effects of glucose on delta cell $[Ca^{2+}]_i$ [11]. It is possible that this is a consequence of the cell isolation procedure and culture, as the glucose-responsiveness of human beta cells is also highly variable (M. Braun, unpublished data). However, it should nevertheless be considered whether glucose, as in mouse delta cells [10], exerts its principal stimulatory effect on somatostatin secretion by a K_{ATP} channel-independent mechanism (cf. [32]). Such an interpretation would be in accord with the relatively weak stimulatory effects of tolbutamide on somatostatin secretion (Fig. 1e). In mouse delta cells this effect involves Ca^{2+}-induced Ca^{2+} release [10]. The presence of a prominent slow component that in some cells was triggered by a delay is suggestive of a similar mechanism in human delta cells. Human islets express both ryanodine and inositol 1,4,5-triphosphate ($InsP_3$) receptors (ESM Fig. 1b). However, in preliminary experiments using ryanodine or Ruthenium Red to inhibit ryanodine receptors, no clear-cut inhibition of the slow component of exocytosis was observed. Thus, the cellular background of the K_{ATP}-channel-independent somatostatin secretion and slow component of capacitance increase remains obscure.

Interestingly, many isolated delta cells remained hyperpolarised even in the presence of high glucose, tolbutamide or Ba^{2+} (cf. Fig. 2h). Thus, it is likely that delta cells are equipped with an additional hyperpolarising conductance, the identity of which remains to be determined. In these cells, robust electrical activity consisting of large overshooting action potentials could be evoked by injection of 5–10 pA of depolarising current. This raises the possibility that in intact pancreatic islets, delta cells are under paracrine regulation and that factors released from neighbouring beta cells depolarise the membrane potential sufficiently to elicit electrical activity. One such factor might be γ-aminobutyric acid (GABA); this neurotransmitter is released from human beta cells in a glucose-dependent manner, and activation of $GABA_A$ receptor Cl^- channels in human delta cells results in membrane depolarisation and action potential firing (M. Braun, R. Ramracheya and P. Rorsman, unpublished data). Regulation of delta cells by factors released from beta cells is also attractive given the evidence suggesting that the loss of adequate insulin secretion in diabetes associates with dysregulation of somatostatin secretion [5, 6].

Acknowledgements This work was supported by the Wellcome Trust, the European Union (Biosim [LSHB-CT-2004-005137] and Eurodia [SHM-CT-2006-518153]), and the UK Department of Health (NIHR Biomedical Research Centres funding scheme). We thank S. Hughes, D. Gray and S. Cross for isolation of human islets, J. Galvanovskis for help with curve fitting, A. Clark for help with

immunohistochemistry and D. Wiggins for assistance with hormone release measurements.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript

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