Paracrine control of α-cell glucagon exocytosis is compromised in human type-2 diabetes

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Glucagon is released from pancreatic α-cells to activate pathways that raise blood glucose. Its secretion is regulated by α-cell-intrinsic glucose sensing and paracrine control through insulin and somatostatin. To understand the inadequately high glucagon levels that contribute to hyperglycemia in type-2 diabetes (T2D), we analyzed granule behavior, exocytosis and membrane excitability in α-cells of 68 non-diabetic and 21 T2D human donors. We report that exocytosis is moderately reduced in α-cells of T2D donors, without changes in voltage-dependent ion currents or granule trafficking. Dispersed α-cells have a non-physiological V-shaped dose response to glucose, with maximal exocytosis at hyperglycemia. Within intact islets, hyperglycemia instead inhibits α-cell exocytosis, but not in T2D or when paracrine inhibition by insulin or somatostatin is blocked. Surface expression of somatostatin-receptor-2 is reduced in T2D, suggesting a mechanism for the observed somatostatin resistance. Thus, elevated glucagon in human T2D may reflect α-cell insensitivity to paracrine inhibition at hyperglycemia.
Glucagon is released from pancreatic α-cells and counteracts the glucose-lowering actions of insulin by stimulating gluconeogenesis and hepatic glucose output. Initially thought of only as part of the body’s defense against hypoglycemia, it is now clear that inadequate glucagon levels also contribute to diabetic hyperglycemia and present a challenge for diabetes management1,2. Glucagon secretion is triggered by low blood glucose and suppressed at physiological glucose levels, and both α-cell intrinsic and paracrine mechanisms have been cited to explain these effects. In the intrinsic models, glucose metabolism and the generation of ATP play a central role3–6, either through subtle, KATP channel-dependent depolarization of the resting membrane potential and subsequent inactivation of Na+–channels7–10, or as consequence of glucose-induced activation of the sarco/endoplasmic reticulum Ca2+-ATPase that leads to closure of store-operated channels and hyperpolarization11,12. In addition, intrinsic glucose-dependent cAMP signaling may play a role13. However, none of these models fully explain the glucose concentration dependence of glucagon secretion, in particular in the hyperglycemic range.

Glucagon secretion is also under paracrine control from neighboring β- and δ-cells, and the inhibitory effects of somatostatin14–18, insulin19–23 and GABA24,25 have long been recognized. Paracrine inhibition is likely to play a role at elevated glucose levels, when β- and δ-cells are active. Indeed, glucagon is secreted in pulses that are anti-synchronous to pulses of insulin and somatostatin26,27. This relationship is important for the postprandial suppression of glucagon secretion and is lost in type-2 diabetes and pre-diabetes28–30. α-cells express the somatostatin receptor SSTR2, which leads to hyperpolarization via activation of GIRK-channels31,32. Somatostatin also inhibits the exocytosis machinery via calcineurin32, and inhibits α-cell exocytosis by effectively decreasing cytosolic cAMP33,34. Insulin receptor signaling is required for the suppression of glucagon secretion in vivo35, but the precise mechanisms behind this are still debated20,34,36. Decreased sensitivity to insulin (or somatostatin) may therefore underlie the inadequate glucagon secretion in type-2 diabetes37.

Glucagon is stored in ~7000 granules (diameter ~270 nm) and secreted by Ca2+- and SNARE protein-dependent exocytosis38,39. At any time, only ~1% of these granules are in a releasable state that can undergo exocytosis upon Ca2+-influx40. Paracrine signaling and glucose regulate glucagon secretion at least in part by affecting the size of this releasable pool of granules32,38,41. In many endocrine cells, secretory granules become release ready by sequential docking at the plasma membrane and assembly of the secretory machinery (priming)42,43. Although disturbances in these steps have been documented in β-cells of type-2 diabetic donors44–47, they have not yet been studied in α-cells. An obstacle for understanding the regulation of α-cells has been the difficulty to isolate intrinsic and paracrine factors of α-cell regulation, as well as species differences between humans and rodent models. Glucagon secretion in vivo and in intact islets is affected by the presence of neighboring cell types, while single-cell electrophysiological measurements are invasive and may not reflect the in vivo situation.

In the current work, we took an optical approach to study glucagon granule exocytosis in α-cells of non-diabetic (ND) and type-2 diabetic (T2D) human subjects. We report that α-cells within intact islets respond with physiological inhibition of exocytosis by elevated glucose, whereas dispersed α-cells have a V-shaped response to glucose due to the lack of paracrine inhibition by insulin and somatostatin from neighboring β- and δ-cells. Importantly, α-cells of T2D are resistant to inhibition by insulin and somatostatin, which might underlie the hyperglucagonemia in type-2 diabetes.

**Results**

**Exocytosis of glucagon granules in human α-cells.** Docking and exocytosis of glucagon granules at the plasma membrane was studied in dispersed islet preparations from 68 non-diabetic (ND) donors that all had glycated hemoglobin HbA1c values <6% (average 5.57 ± 0.29%, Supplementary Fig. 5). To identify α-cells, we transduced with Pppg-EFGP and the secretory granule marker NPY-mCherry, or with Pppg-NPY-EFGP (Fig. 1a and S1a, b) to drive expression of fluorescent proteins from the pre-proglucagon promoter (see methods). After culture for 26–48 h, α-cells were imaged by total internal reflection (TIRF) microscopy, which selectively images fluorescence near the plasma membrane (exponential decay constant τ ~ 0.1 μm). The granule marker had a punctate staining pattern and excellent overlap with anti-glucaagon immunostaining (Fig. 1a). Local application of elevated K+ (75 mM, replacing Na+) to depolarize the cells resulted in exocytosis, seen as rapid disappearance of individual fluorescently labeled granules (gr, see Fig. 1b and examples in S1B,C). Exposure to elevated K+ for 40 s released 0.078 ± 0.004 granules μm–12 (169 α-cells/29 ND donors, Fig. 1c, black). Exocytosis proceeded initially with a burst (5.2 × 10–3 gr μm–2 s–1 during the first 10 s) and decreased later to <0.6 × 10–3 gr μm–2 s–1; these rates are about one-third of those observed in human β-cells44. Fitting the cumulative exocytosis (n = 1530 granules) with a double exponential function revealed two components with time constants of τ = 3.6 ± 0.2 s and 19.9 ± 0.9 s (Fig. 1c). The faster component made up 39 ± 3% of the total response, and likely corresponds to the RRP. Exocytosis occurred in granules that from the start of the experiment had been docked at the plasma membrane. We therefore quantified changes in docked granules during the experiment, by measuring the density of granules in the TIRF field. Stimulated exocytosis partially depleted docked granules (Fig. 1d), indicating that replacement by docking of new granules is relatively slow (a notion confirmed by a double stimulation protocol, Supplementary Fig. 2). On average, 13 ± 0.7% of the docked granules were released during the stimulation. Thus, depolarization of α-cells results in exocytosis with biphasic kinetics similar to those in other endocrine cells, indicating the existence of granule pools with differing release probabilities.

**Reduced granule docking and exocytosis glucagon in T2D α-cells.** During the course of this study, we also received islets from 21 donors that had been clinically diagnosed with type-2 diabetes (T2D), or whose glycated hemoglobin (HbA1c) values were above 6% (average 6.6 ± 0.7%, Supplementary Fig. 5). In dispersed T2D α-cells, K+–stimulated exocytosis was reduced to 66 ± 8% of that in ND α-cells (p = 0.004, 0.052 ± 0.005 gr μm–2; 75 cells/12 donors; Fig. 1b, c), mostly due to a reduced amplitude of the fast component (τ = 2.1 ± 0.1 s, 25 ± 3%, n = 441 granules, p = 0.0008 vs ND). Docked granules were slightly fewer in T2D α-cells (0.57 ± 0.02 gr μm–2, 106 cells/17 donors) compared with ND (0.61 ± 0.008 gr μm–2; p = 0.01, 399 cells/50 donors, Fig. 1d). Exocytosis and granule density correlated on a per-donor basis (Pearson r = 0.42, p = 0.006, 41 donors, Fig. 1e), and both exocytosis (r = 0.49, p = 0.002; Fig. 1f) and docked granules (r = 0.37, p = 0.003; Fig. 1g) anti-correlated with the donor’s HbA1c, as is the case in human β-cells44. The relationships are surprising given that reduced glucagon secretion should lead to reduced blood glucose and HbA1c values, and suggest that the diabetic state is causal for the reduced release capacity of T2D α-cells.

**Voltage-dependent currents are normal in T2D α-cells.** Since exocytosis in α-cells depends on Ca2+-influx, we characterized voltage-dependent ion currents using patch-clamp...
electrophysiology. Dispersed ND or T2D α-cells were voltage clamped in whole-cell mode, and subjected to step depolarizations up to +70 mV from a holding potential of −70 mV (Fig. 2a). Analysis of the resulting inward currents revealed peak Ca\(^{2+}\) and Na\(^{+}\)-currents (Fig. 2c) that were of similar amplitude in ND and T2D cells. Half-maximal Ca\(^{2+}\)-current activation was reached at −23 ± 0.4 (ND) and −25 ± 1.4 mV (T2D, n.s.), and half-maximal Na\(^{+}\)-current activation was at −25 ± 0.6 (ND) and −24 ± 0.8 mV (T2D, n.s.). We also determined depolarization evoked membrane capacitance increases, a measure of exocytosis (Fig. 2d, e). A train of 14 depolarizations to 0 mV lasting 200 ms each resulted in a total capacitance increase of 112 ± 19 fF in ND cells and 78 ± 19 fF in T2D cells. This corresponds to a reduction of exocytosis by 25 ± 10% in T2D (p = 0.1), which is similar to the reduction observed by imaging granule release (Fig. 1d). Cell size, as assessed by cell capacitance, was not different in the two groups (Fig. 2f). Thus, reduced exocytosis in T2D α-cells cannot be explained by changes in Ca\(^{2+}\)-channel behavior.

Glucose regulation of glucagon secretion. Next, we determined the physiological glucose dependence of dispersed α-cells by measuring spontaneous exocytosis in a range of ambient glucose concentrations (1, 3, 7, 10, or 20 mM; at least 20 min pre-incubation), without imposing any depolarization. In movies lasting 3 min, we quantified granule exocytosis, docked granules, and the rate of docking of new granules at the plasma membrane (Fig. 3a–d). Spontaneous exocytosis was observed in all glucose concentrations, with a bimodal (V-shaped) dose response to glucose. Inhibition was about half at the nadir of 7 mM glucose, and ND and T2D cells behaved essentially identically (Fig. 3b).
Docked granules, and to a lesser degree the rate of docking (in the same cells) likewise had a bimodal response to glucose, with a nadir at 7 mM (Fig. 3a, c, d). In separate experiments, we noticed that the response to changes in the glucose concentration was slow, and only minor during 2 min observation (Supplementary Fig. 3a, b). Thus, the glucose dependence of dispersed α-cells does not reflect physiological glucagon secretion, and exocytosis is accelerated in the hyperglycemic range instead of being inhibited. The data also suggest that the availability of docked granules is part of the regulation of exocytosis in α-cells.

To experimentally isolate direct glucose effects on the exocytosis machinery, we applied K+-stimulations to α-cells bathed in 1, 7, or 10 mM glucose (Fig. 3e). This approach primarily elicits exocytosis of granules that are “primed” for exocytosis, the readily releasable pool (RRP). At all glucose concentrations, elevated K+-induced exocytosis was reduced by about half compared with 1 mM glucose (−45 ± 9%, p = 8 × 10−4, 30 cells/6 donors), or 10 mM glucose (−47 ± 6%, p = 5 × 10−6, 71 cells/14 donors). This reduction was most prominent during the initial burst phase, which may correspond to the immediately releasable pool of granules in β-cells. At all glucose concentrations, K+-induced exocytosis was significantly slower in T2D α-cells than in ND, but the bimodal glucose dependence of docking and exocytosis was preserved (Fig. 3f).

We conclude that the α-cell exocytosis machinery is regulated by the ambient glucose concentration.

**Exocytosis in α-cells within intact islets.** The unexpected V-shaped glucose response of dispersed α-cells indicates that intrinsic regulation cannot explain the physiological inhibition of glucagon secretion in hyperglycemia. An alternative are paracrine effects from neighboring β- and δ-cells, which prompted us to quantify glucose dependent exocytosis in α-cells within intact islets (Fig. 4a, b). As expected, exocytosis of α-cells in intact islets varied with the glucose concentration, with inhibition in 7 mM glucose by 67 ± 15% (p = 0.016; 10 islets/3 donors), compared with 1 mM glucose (11 islets/3 donors). Exocytosis was also inhibited in 10 mM (by 56 ± 16% vs 1 mM, p = 0.024, 16 cells/4 donors), in contrast to dispersed α-cells. However, this inhibition was prevented by the SSTR2-specific somatostatin receptor antagonist CYN154806 (200 nM in the bath solution; p = 0.003 vs 10 mM glucose, 19 cells/3 donors). Similarly, block of insulin action with the insulin receptor antagonist S961 (1 μM) prevented inhibition of exocytosis at 10 mM glucose (p = 0.003 vs 10 mM glucose, 11 cells/2 donors), indicating that paracrine signaling is required for proper glucagon control in hyperglycemic conditions. In intact islets of T2D donors, α-cell exocytosis had a bimodal dose response to glucose that lacked inhibition hyperglycemia (Fig. 4c). At 7 mM glucose (16 islets/4 donors), exocytosis was inhibited to about half compared with 1 mM (21 islets/5 donors), whereas 10 mM glucose has no effect (29 islets/5 donors). Thus, α-cells within intact ND islets have a physiological response to glucose. Disruption of paracrine signaling by antagonists or islet dispersion leads to a V-shaped glucose response, similar to that observed in intact T2D islets.

We confirmed expression of SSTR2 in human α-cells by co-immunostaining the receptor and glucagon in pancreatic sections of 10 human donors (5 ND, 5 T2D; Fig. 4d). In ND islets, SSTR2 distribution was mostly confined to the cell membrane of α- and other islet cells, whereas in T2D islets the SSTR2 staining was both weaker and largely vesicular (Fig. 4d). Quantitative analysis confirmed this conclusion and estimated that SSTR2 surface
expression is decreased by 44 ± 7%, in T2D (824 cells/5 T2D donors vs 828 cells/5 ND donors Fig. 4e). Glucagon levels and distribution were similar in both groups (Fig. 4f). Insensitivity to somatostatin is therefore the result of excessive receptor internalization, as has recently been shown for pituitary cells.

Paracrine regulation of exocytosis in dispersed α-cells. Glucagon secretion is regulated by a network of paracrine mechanisms, some of which act directly on α-cells. We therefore quantified K⁺-stimulated exocytosis dispersed α-cells in presence of a panel of islet paracrine effectors (somatostatin (SST, 400 nM), insulin (INS, 100 nM), forskolin (FSK, 2 µM), γ-aminobutyric acid (GABA, 400 nM), adrenaline (ADR, 5 µM), or glutamate (Glut, 1 mM), all present in the bath) at 1 or 10 mM glucose (Fig. 5). In 10 mM glucose (Fig. 5a), the δ-cell hormone somatostatin inhibited K⁺-stimulated exocytosis by 65 ± 4% (p = 2 x 10⁻⁶, 53 cells/9 donors, vs control 71 cells/14 donors). β-cell factors likewise inhibited exocytosis, with insulin reducing it by 53 ± 5% (p = 8 x 10⁻⁵, 53 cells/8 donors) and GABA reducing it by 24 ± 13% (n.s., 14 cells/3 donors). In contrast, adrenaline doubled (p = 4 x 10⁻⁹, 30 cells/5 donors) and glutamate tripled α-cell exocytosis (p = 14 x 10⁻²⁰, 16 cells/3 donors). Elevated cAMP, after exposure to forskolin, had no effect on exocytosis (n.s., 30 cells/5 donors), in contrast to previous reports. In α-cells of T2D donors (Fig. 5b), adrenaline accelerated exocytosis about three fold (p = 6 x 10⁻⁹; 19 cells/3 donors). In contrast, the inhibition by somatostatin or insulin was lost in T2D. None of the tested compounds affected the density of docked granules (Fig. 5a, b lower), suggesting that paracrine factors modulate α-cell exocytosis by affecting granule priming, rather than docking.

In hypoglycemic conditions (1 mM glucose, Fig. 5c, d), neither somatostatin (27 cells/6 donors, blue) nor insulin (24 cells/5 donors, green) affected K⁺-stimulated exocytosis of dispersed α-cells, while adrenaline (p = 2 x 10⁻⁶, 15 cells/3 donors, pink) and glutamate (p = 9 x 10⁻¹⁰, 9 cells/2 donors, orange) accelerated exocytosis 2–3-fold compared with control (1 mM glucose, 30 cells/6 donors). T2D α-cells (Fig. 5d) behaved identical to ND α-cells with regard to somatostatin (17 cells/3 donors, blue), insulin (23 cells/3 donors, green) and adrenaline (p = 2 x 10⁻¹², 10 cells/2 donors, pink), except for a moderate reduction of exocytosis at 1 mM glucose. No differences in the density of docked granules were observed in presence of any of the effectors, or comparing T2D with ND cells (Fig. 5c, d lower).

Rapid paracrine inhibition by insulin and somatostatin. Glucagon secretion oscillates with a frequency of minutes, which is inconsistent with the relatively slow glucose dependent regulation (Supplementary Fig 3). We therefore determined the time course of paracrine inhibition of spontaneous exocytosis by rapidly applying somatostatin (Fig. 6a, b, blue shading) or insulin (Fig. 6c, d, green shading) to dispersed α-cells. Maximal inhibition by somatostatin was reached within a few seconds (mono-
Somatostatin inhibit both exocytosis (Fig. 5) and electrical activity or electrical activity (Fig. 6g, h). In summary, insulin and pulses had little effect on the time course of exocytosis (Fig. 6b, d) while glucagon pulses impaired exocytosis by decreasing the dynamic range (Fig. 6a). Both effects are lost in T2D, which is consistent with the notion that these cells are resistant to paracrine inhibition.

Discussion

Glucose controls glucagon secretion by intrinsic and paracrine mechanisms, but their relative significance is still debated, and secretory defects in type-2 diabetes are not well understood. The current work is first in using high-resolution microscopy to study glucagon secretion both in intact islets and in single dispersed α-cells of healthy and type-2 diabetic donors, thus isolating intrinsic from paracrine mechanisms while having full control over paracrine signaling. We show that in the absence of paracrine influence, isolated α-cells respond appropriately to hypoglycemia with an increase in glucagon granule exocytosis. This is consistent with the glucose dependence of glucagon secretion from intact islets (but not FACs sorted α-cells) and indicates that glucagon secretion in the lower glucose-concentration range is mostly under intrinsic control. With only 2-fold difference in the exocytosis rate between minimal secretion at 7 mM and maximal secretion at 1 mM glucose, the dynamic range is small compared with β-cells. Surprisingly, exocytosis of dispersed α-cells is stimulated in the hyperglycemic range, leading to an unphysiological V-shaped response with maximal exocytosis above 10 mM glucose. This is in contrast to intact islets, in which glucagon secretion is depressed between 3 and 20 mM glucose. We confirm this here by exocytosis measurements in intact islets.
Fig. 5 Paracrine regulation of exocytosis in dispersed α-cells. a Cumulative time course (upper), total exocytosis (middle), and initial density of docked granules (lower) during K⁺-stimulated (gray bar) exocytosis in dispersed ND α-cells in control conditions (black, 10 mM glucose, n = 71 cells/14 donors) or exposed to somatostatin (light blue, SST, 400 nM, n = 30 cells/5 donors), GABA (brown, 400 nM, n = 14 cells/3 donors), adrenaline (pink, ADR, 5 μM, n = 30 cells/5 donors), glutamate (orange, Glut, 1 mM, n = 16 cells/3 donors). In a-d, significant differences compared with control are indicated with p-values (one-way ANOVA, Fisher posthoc test). Data are presented as mean values ±SEM. b As in A, but for dispersed T2D α-cells. T2D ctrl n = 33 cells/6 donors, T2D SST n = 26 cells/5 donors, T2D INS n = 19 cells/4 donors, T2D ADR n = 19 cells/3 donors. c, d As in a, b, but in presence of 1 mM glucose. ND ctrl n = 30 cells/6 donors, ND SST n = 27 cells/6 donors, ND INS n = 24 cells/5 donors, ND ADR n = 15 cells/3 donors, ND Glut n = 9 cells/2 donors, T2D ctrl n = 27 cells/5 donors, T2D SST n = 17 cells/3 donors, T2D INS n = 23 cells/3 donors, and T2D ADR n = 10 cells/2 donors.

and provide evidence that this depression depends on the inhibitory effects on insulin and somatostatin that are released by neighboring β- and δ-cells. Evidently, α-cell intrinsic mechanisms are sufficient for the regulation of glucagon secretion hypo- and normoglycemic range (0–7 mM glucose), while paracrine inhibition is responsible for the physiological response in the hyperglycemic range. Consequently, appropriate glucagon secretion in the hyperglycemic range is lost when α-cells are removed from their context within the islet.

Elevated glucagon is a hallmark of type-2 diabetes. Despite this, both glucose-dependent and depolarization (K⁺)-induced exocytosis was reduced in α-cells from donors that had been diagnosed with T2D. Both exocytosis and docked granules were moderately anti-correlated with donor HbA1c values. This indicates that the exocytosis machinery in α-cells from type-2 diabetics is slightly impaired, while electrophysiological parameters (that determine electrical activity, depolarization and Ca²⁺-influx) were normal. The reason for this is unknown, but may reflect reduced expression of certain exocytosis-related proteins, as is the case in β-cells. The reduced exocytotic capacity in T2D α-cells is unrelated to changes in electrical activity, because it could be observed in K⁺-stimulation experiments in which the membrane potential is clamped. Since exocytosis in single α-cells is impaired rather than increased in T2D, the hyperglucagonemia in diabetic humans must be due to mechanisms that are lost in isolated cells, such as paracrine or neuronal regulation. In addition, gut derived glucagon may contribute to hyperglucagonemia following oral glucose intake.

Strikingly, the inhibitory effects of insulin and somatostatin on glucagon exocytosis were strongly reduced in cells from T2D donors, in parallel with internalization and reduced surface expression of SSTR2, the major somatostatin receptor in human α-cells. This points to an α-cell resistance to insulin and somatostatin as the main cause for inadequate glucagon secretion in type-2 diabetes, which in turn exacerbates hyperglycemia. Insulin resistance is a hallmark of T2DM, and has previously been proposed as mechanism for hyperglucagonemia. For example, insulin resistance is associated with fasting glucagon levels, and this inverse relationship is lost in type-2 diabetes. Interestingly, SSTR2 surface expression was also reduced in β-cells within T2D islets, suggesting that reduced somatostatin sensitivity may contribute also to increased insulin secretion, as observed early during the development of T2D. While there is reason to believe that this is a consequence of altered δ-cells activity, its role may be to adapt islets to periods of greater food availability.

Exposure to insulin, somatostatin, and GABA reduced α-cell exocytosis, while adrenaline and glutamate stimulated it. This is consistent with the known effects of these signaling molecules on islets, as well as systemically. We show here that these effects are
very rapid (seconds), which is consistent with the frequency of pulsatile glucagon release in vivo and from intact islets. In the absence of glucose-dependent control, paracrine inhibition by insulin and somatostatin is therefore the most likely mechanism for glucagon regulation in the hyperglycemic range. It can be speculated that the differential glucose dependence of insulin and somatostatin secretion is reflected in different target glucose ranges for their action on α-cells. All tested paracrine modulators affected exocytosis machinery at the priming step, rather than by increasing granule docking. This is consistent with previous findings that somatostatin inhibits exocytosis in rat α-cells through Gi-dependent depriming32, and reports that antagonists of SSTR212 or the associated G-protein cascade12 increase glucagon secretion without altering the glucose-dependent inhibition of glucagon secretion. We did not observe any bursts of exocytosis, as might be expected given the pulsatile glucagon secretion from intact islets. This is in line with the absence of membrane potential oscillations in single cells61 (that we confirm here), and indicates that the islet context is required not just for intra-islet synchronization, but for oscillatory α-cell behavior as such.

Capacitance measurements indicate that glucagon granules exist in at least two states with different release probabilities, which are often referred to as the readily releasable pool of granules (RRP) and a larger reserve pool (RP)38,62. We show here that glucagon granules were present at the plasma membrane for extended periods before undergoing exocytosis. We interpret this as the relatively slow conversion from RP to RRP that reflects the molecular assembly of the secretory machinery at the release site, in analogy with the situation in β-cells42,44. Throughout the glucose range, the rate of exocytosis was nearly identical to that of granule docking (Fig. 1b–d), suggesting that docking is rate limiting for secretion. This may indeed be the case during strong (non-physiological) stimulation, as illustrated by the finding that the glucose-dependence of depolarization-induced exocytosis followed that of granule docking. However, in physiological conditions elevated K+ accelerated exocytosis ~50-fold (during the first second), which indicates a large excess in exocytotic capacity that is not triggered by normal α-cell electrical activity. A possible explanation could be that only a limited number of granules is positionally primed, i.e. located near voltage-gated Ca2+-granules63. Further theoretical
work is required to understand the combination of factors affecting granule exocytosis, and the granule conversion rates provided here may be useful in this regard.

**Methods**

**Tissue.** Pancreatic islets and pancreas sections were obtained from human cadaveric donors by the Nordic Network for Clinical Islet Transplantation Uppsala64 (ethical approval by Uppsala Regional Ethics Board) or the ADI Isletcore at the University of Alberta65 (ethical approval by Alberta Human Research Ethics Board, Pro00001754), with written donor and family consent for use in research. With human tissue complied with all relevant ethical regulations for use of human tissue in research and the study was approved by the Uppsala Regional Ethics Board (2014/204-31/4). Isolated islets were cultured free-floating in sterile dishes in CMRL 1066 culture medium containing 5.5 mM glucose, 10% fetal calf serum (FCS), 2 mM l-glutamine, streptomycin (100 U/ml), and penicillin (100 U/ml) at 37 °C in an atmosphere of 5% CO2 up to 2 weeks. Islets were dispensed into single cells by gentle pipetting in cell dissociation buffer (Thermo Fisher Scientific) supplemented with trypsin (0.005%, Life Technologies). Cells were then washed and plated in serum-containing medium onto 22-mm polylysine-coated coverslips, allowed to settle overnight, and then transduced using adenovirus. In Fig. 4a, c, the dispersion step was omitted and intact islets were transduced with Ppyp-NPY-EGFP adenovirus and allowed to settle onto 22-mm polylysine-coated coverslips.

**Labeling of human pancreatic a-cells and glucagon granules.** To identify a-cells, we transduced cells with adenovirus coding for enhanced green fluorescent protein (EGFP) under the control of the pre-proglucagon promoter66. The system takes advantage of Tet-On conditional expression in the presence of 4 μM doxycycline, to drive expression of EGFP. The cells were simultaneously transduced with adNPY-mCherry, a well-established secretory granule marker. Alternatively, adenovirus coding for EGFP-tagged neuropetide Y under control of the pre-proglucagon promoter (Pppg-EGFP) was used, thus combining the identification of a-cells and secretory granule label. For both approaches, immunostaining with an anti-glucagon antibody confirmed that over 90% of the fluorescently labeled cells were a-cells (Fig. 1a and Supplementary Fig. 1a). Approximately one-third of the glucagon positive cells were labeled with Ppyp-NPY-EGFP (Supplementary Fig. 1a). NPY-EGFP labeled granules had excellent overlap with punctate glucagon staining (Fig. 1a), with 94 ± 1% of glucagon positive granules being labeled with NPY-EGFP (37 cells, 5 donors). We verified that exocytosis in the identified cells was stimulated by adrenaline (Supplementary Fig. 1b–d), which increases intracellular Ca2+ in α- but not β-cells.

**TIRF microscopy.** Cells were imaged using a custom-built lens-type total internal reflection (TIRF) microscope based on an AxiosObserver Z1 with a x100/1.45 objective (Carl Zeiss). Excitation was from two DPPS lasers at 491 and 561 nm. Total internal reflection microscopy was done with a Zeiss LSM 780 confocal microscope using a 63/1.40 objective (Carl Zeiss). Excitation was from two DPSS lasers at 491 and 561 nm. Tissue Methods

**Granule exocytosis, and the granule conversion rates provided here may be useful in this regard.**

**Image analysis.** Exocytosis events were identified based on the characteristic rapid loss of the granule marker fluorescence (1–2 frames). Granule docking events were rare and defined as granules that approached the TIRF field and becoming laterally confined once they reached their maximum brightness66. Docked granules were counted using the ‘find maxima’ function in ImageJ. Values were normalized to each cell’s contact area with the coverslip footprint. The AF parameter estimates the fluorescence that is specifically localized to a granule, but subtracting a local background value (average of a 5 pixel wide annulus) from the average fluorescence value in a 3 pixel wide circle, both centered at the granule position.

**Immunostaining of pancreatic sections.** For analysis of SSTR2 expression, deparaffinized human pancreatic tissue sections (biobank samples obtained from the EXODIAB consortium, Uppsala) were heated in a buffer containing 10 mM Tri-sodium citrate and 0.05%Tween 20 (pH 6) for 15 min, allowed to cool, and rinsed with Dako wash buffer 1x. After a 30-min blocking step (Background Sniper, Biocare Medical), sections were rinsed with wash buffer 1x (Dako) and incubated with anti-SSTR2 (Abcam ab54152, diluted 1:500 in wash buffer), and anti-glucagon antibodies (Dako A0565, diluted 1:500 in wash buffer) overnight at 4°C. The slides were then washed in wash buffer and incubated with fluorophore-labeled secondary antibodies (diluted in Dako wash buffer 1x) for 30 min at room temperature. Fluorescence was visualized using a Zeiss LSM 780 confocal microscope. For analysis, 3–pixel wide linescans of fluorescent intensity were calculated as illustrated in Fig. 3e, f. Top. Background subtracted and estimated as the minimum value to the left of the alignment point (corresponding to the nucleus location), after 3 x 3 median filtering.

**Electrophysiology.** Standard whole-cell voltage clamp and capacitance recordings were performed using an EPC-9 patch amplifier (HEKA Electronics, Lambrecht/Pfalz, Germany) and PatchMaster software. Voltage-dependent currents were investigated using an IV-protocol, in which the membrane was depolarized from −70 mV to +80 mV (10 mV steps) during 50 ms each. Currents were compensated for capacitive transients and linear leak using a P/4 protocol. Exocytosis was detected as changes in cell capacitance using the lock-in module of Patchmaster (30 mV peak-to-peak with a frequency of 1 kHz).

**Statistics.** Data are presented as mean ± SEM unless otherwise stated. Statistical significance was tested using t-test for comparing ND and T2D groups, or ANOVA for multiple comparisons, as stated (in Origin 2018). Correlation was quantified as Pearson coefficient r using Excel.

**Data availability**

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

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Author contributions

M.O.H. and S.B. designed experiments and analyzed the data. M.O.H. performed experiments. P.E.L. performed electrophysiology experiments. M.O.H. and N.R.G. prepared human islets for imaging. M.O.H. designed and generated the Pppg-NPY-GFP virus construct. A.T. supplied Pppg-GFP plasmid and adenovirus. S.B. conceived the study. S.B. and M.O.H. wrote, and all authors critically reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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