Synaptotagmin-7 as a positive regulator of glucose-induced glucagon-like peptide-1 secretion in mice

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Abstract

Aims/hypothesis Glucagon-like peptide-1 (GLP-1), a hormone with potent antihyperglycaemic effects, is produced and secreted from highly specialised gut endocrine L-cells. It regulates glucose homeostasis by promoting glucose-dependent insulin secretion, suppressing glucagon secretion and enhancing insulin sensitivity. Similar to islet alpha and beta cells, L-cells are electrically excitable, and express calcium channels and ATP-sensitive potassium channels. GLP-1 is also stored in secretory granules, the exocytosis of which is triggered by increased intracellular calcium levels. Although the calcium dependence of GLP-1 granule exocytosis is well established, the identities of calcium-sensing proteins in

GLP-1 secretion remain elusive. Here we tested whether synaptotagmin-7, a calcium sensor in pancreatic alpha and beta cells, regulates GLP-1 secretion.

Methods We studied GLP-1 secretion using synaptotagmin-7 knockout (KO) mice and GLUTag cells with lentiviral-mediated synaptotagmin-7 silencing.

Results We found that synaptotagmin-7 was co-localised with GLP-1 in intestinal L-cells. GLP-1 secretion was impaired in synaptotagmin-7 KO mice when they were challenged by glucose ingestion. Lentiviral knockdown (KD) of synaptotagmin-7 in GLUTag cells led to similar reductions in GLP-1 secretion, as determined by biochemical assays and by membrane capacitance measurements. Calcium response was not altered in synaptotagmin-7 KD cells.

Conclusions/interpretation These results demonstrate that synaptotagmin-7 functions as a positive regulator of GLP-1 secretion in intestinal L-cells and GLUTag cells, consistent with its proposed role as a calcium sensor in GLP-1 secretion.

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Abbreviations

GLP-1	Glucagon-like peptide 1
KD	Knockdown
KO	Knockout
KRH	Krebs–Ringer HEPES
shRNA	Short hairpin RNA

Introduction

Glucagon-like peptide-1 (GLP-1), encoded by the proglucagon gene, is produced and secreted from gut endocrine L-

cells in ileum and colon of the distal gut [1–3]. GLP-1 is one of the two major incretins, which have attracted great attention in recent years due to their beneficial effects on glucose and energy homeostasis [4]. GLP-1 regulates glucose homeostasis at multiple sites of action, e.g. by augmenting glucose-dependent insulin secretion, suppressing glucagon secretion and improving insulin sensitivity [1, 4]. In addition, GLP-1 can also reduce appetite and food intake [1, 4]. Considering the intimate link between obesity and diabetes, GLP-1 appears to be an ideal target for treatment options for both conditions. Since GLP-1 has a very short half-life, current strategies in GLP-1-based drug development include modified GLP-1 or GLP-1 analogues with longer half-life, and inhibitors of dipeptidyl peptidase-4, the enzyme that cleaves and inactivates GLP-1 [4]. An alternative option may be to target GLP-1 secretion regulation to fully utilise endogenous GLP-1 production and secretion potential of L-cells. Although progress has been made in understanding the regulation of GLP-1 secretion, many fundamental questions remain unanswered [1, 5–7].

Similar to other endocrine cells, intestinal L-cells store GLP-1 in secretory granules and release it through regulated exocytosis in response to increased intracellular calcium level [3, 8]. L-cells express calcium channels and ATP-sensitive potassium channels, and like islet alpha cells, they are electrically excitable [3, 9]. Nutrient, neural and paracrine factors act through different pathways to induce GLP-1 granule exocytosis from L-cells by a common mechanism, i.e. the increase of intracellular calcium levels [1, 3, 10, 11]. By analogy to insulin secretion from pancreatic beta cells, glucose, the main stimulator of GLP-1 release, was proposed to stimulate GLP-1 granule exocytosis by a series of metabolic and membrane events that include increased ATP production, closure of ATP-sensitive potassium channels, opening of voltage-dependent calcium channels and elevation of cytoplasmic calcium level [3, 7]. In addition, glucose may stimulate GLP-1 secretion by directly depolarising L-cell plasma membrane through the electrogenic action of sodium-glucose cotransporter and the consequent opening of calcium channels [6]. Although it is well established that GLP-1 release is triggered by increased calcium levels, it is not clear which proteins are responsible for sensing the calcium rise to trigger GLP-1 granule fusion with plasma membrane in L-cells.

Several members of the synaptotagmin family have been shown to function as calcium sensors in neurotransmitter and hormone release [8, 12–17]. In particular, synaptotagmin-7 regulates calcium-dependent insulin secretion in pancreatic beta cells, glucagon secretion in alpha cells and catecholamine release in chromaffin cells [15–17], and is probably the most important synaptotagmin in hormone secretion

regulation. Based on the functional similarity between these cell types and L-cells, we used synaptotagmin-7 knockout (KO) mice and GLP-1-secreting murine cell line GLUTag cells with synaptotagmin-7 knockdown (KD) in this study to test whether synaptotagmin-7 is involved in the regulation of GLP-1 secretion.

Methods

Animal welfare Synaptotagmin-7 KO mice were generated as previously described [18]. All experiments involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of Agency for Science, Technology and Research, Singapore (numbers 080351 and 090428).

GLP-1 secretion in vivo For oral GLP-1 secretion test, 12–14-week-old mice were fasted for 16–18 h with free access to water. Plasma GLP-1 levels were tested before and 15, 30 and 60 min after ingestion of glucose solution given by gavage at 5 g/kg of body weight (50 µl of blood taken per time point). The blood was mixed with 2 µl 0.5 mol/l EDTA and 10 mg/ml diprotin on ice and centrifuged at 10,000×g for 5 min. Plasma GLP-1 levels were determined by GLP-1 active ELISA (Millipore, St. Charles, MO, USA).

Cell culture and lentivirus-mediated silencing of synaptotagmin-7 in GLUTag cells GLUTag cells were grown in DMEM (11885; Invitrogen, New York, NY, USA) supplemented with 10% (vol./vol.) fetal bovine serum and 1% (vol./vol.) penicillin/streptomycin. The cells were plated in 12-well culture plates, and infected with synaptotagmin-7 short hairpin RNA (shRNA) lentiviral particles (sc-41320-V; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and control shRNA lentiviral particles (sc-108080) according to the manufacturer's protocol. Cells were then selected by 5 µg/ml puromycin (Santa Cruz Biotechnology).

Immunostaining Immunolabelling was performed on frozen mouse ileal sections and GLUTag cells using rabbit antibody against synaptotagmin-7 (Synaptic Systems, Göttingen, Germany) and goat antibody against GLP-1 (Santa Cruz Biotechnology), followed by fluorescence-conjugated secondary antibodies, Alexa Fluor 488 donkey-anti-rabbit IgG and Alexa Fluor 555 donkey-anti-goat IgG (Invitrogen). Digital images of the sections were acquired on a confocal laser-scanning microscope (A1R; Nikon, Tokyo, Japan).

RNA extraction and quantitative PCR Total RNA was extracted from GLUTag cells using a Trizol method

(Invitrogen), treated with DNase I (Roche, Mannheim, Germany) and reverse-transcribed with RevertAid (Fermentas, Vilnius, Lithuania). Quantitative real-time PCR was performed using Taqman assays for mouse synaptotagmin-7 (Mm00444496_ml) and glucagon (Mm00801714_ml) on a real-time PCR System (StepOnePlus; Applied Biosystems, Foster City, CA, USA). β -Actin from a probe set (Mouse ACTB 20 $\times$ VIC-MGB; Invitrogen) was used as endogenous reference.

Measurement of GLP-1 secretion from GLUTag cells Basal medium for cell handling was Krebs–Ringer HEPES (KRH) buffer containing in mmol/l: 130 NaCl, 4.7 KCl, 1.2 KH_2PO_4 , 1.2 MgSO_4 , 2.56 CaCl_2 , 20 HEPES supplemented with 1 mg/ml BSA and no glucose. GLUTag cells were plated in 12-well plates 1 day before the experiment to reach 60% to 80% confluence. After pre-incubation in basal KRH buffer for 2 h, cells were washed and incubated for 30 min in 0.5 ml basal KRH buffer supplemented with 10 mg/ml diprotin. The medium was collected for determination of basal GLP-1 secretion. Next KRH buffer containing 20 mmol/l glucose was added and cells were incubated for another 30 min. Collected buffer was kept on ice and centrifuged to remove floating cells. GLP-1 concentration in the buffer was determined by GLP-1 active ELISA (Millipore). GLP-1 secretion was expressed as percentage of basal release.

Electrophysiology measurements Synaptotagmin-7 KD and control GLUTag cells were plated on coverslips 1 day before experiment. Membrane capacitance was recorded in individual cells within small clusters using the standard whole-cell patch-clamp technique [19, 20]. Exocytosis was induced by a 500 ms depolarising pulse from -70 to 0 mV. Pipette resistance ranged between 3 and 5 M Ω when pipettes were filled with intracellular solution containing (in mmol/l): 117 KCl, 1 CaCl_2 , 2 MgCl_2 , 10 EGTA, 5 MgATP and 10 HEPES (pH 7.2 with KOH). Extracellular solution contained (in mmol/l): 138 NaCl, 5.6 KCl, 2.6 CaCl_2 , 1.2 NaH_2PO_4 , 4.2 NaHCO_3 , 1.2 MgCl_2 , 1 D-glucose and 10 HEPES (pH 7.4, with NaOH). Cells were stimulated at low frequency (<0.05 Hz) to allow full recovery of exocytotic capacity between pulses. Measurements were performed using EPC10 plus patch clamp amplifier and PatchMaster software (HEKA Instruments, Bellmore, NY, USA). Exocytosis was detected as changes in cell membrane capacitance, which was estimated by the Lindau–Neher technique implementing the ‘Sine + DC’ feature of the lock-in module [21]. The amplitude of the sine wave was 10 mV and the frequency was set at 1 kHz. All cell membrane capacitance measurements were performed at 30–32°C.

Calcium measurements GLUTag cells plated on coverslips as described above were loaded with 1 $\mu\text{mol/l}$ Fura-2 in basal KRH buffer for 40 min at 37°C. Coverslips were mounted at the bottom of an open chamber under continuous perfusion of KRH buffer at 37°C [22]. Cells were stimulated with KRH buffer containing 20 mmol/l glucose for 30 min and the Ca^{2+} response was recorded from individual GLUTag cells within small clusters on an inverted microscope (Eclipse TE2000-U; Nikon) using an image analysis system (Metafluor; Molecular Devices, Sunnyvale, CA, USA). Fura-2 was excited at 340 and 380 nm with Lambda DG4 system (Sutter Instrument, Novato, CA, USA). After passing a bandpass filter (535DF35; Omega Optical, Brattleboro, VT, USA), the emitted signal was projected onto a CCD camera (CoolSNAP HQ2; Photometrics, Tuscon, AZ, USA) and the digital images collected. $[\text{Ca}^{2+}]_i$ was calculated from the ratio of 340 and 380 nm signals after background subtraction using the equation described by Grynkiewicz et al. [23], with a K_d of 224 nmol/l.

Western blot Synaptotagmin-7 KD and control GLUTag cells were lysed and protein was quantified using a protein assay (DC; Bio-Rad, Hercules, CA, USA). Samples were loaded and resolved on a 12% (wt/vol.) SDS-polyacrylamide gel and then transferred to a nitrocellulose membrane. The membranes were incubated overnight at 4°C with rabbit polyclonal synaptotagmin-7 antibody (Synaptic Systems) or mouse monoclonal β -actin antibody (Santa Cruz Biotechnology). After incubation with horseradish-conjugated anti-rabbit IgG (GE Healthcare, Piscataway, NJ, USA), the ECL chemiluminescent system (GE Healthcare) was used for signal detection.

Statistical analysis The data are presented as means $\pm$ SEM. Comparisons of data were made using the two-tailed Student’s *t* test with significance limit set at $p<0.05$.

Results

Detection of synaptotagmin-7 in intestinal L-cells We first examined by immunohistochemistry whether synaptotagmin-7 was produced in GLP-1-secreting L-cells. L-cells were identified by GLP-1 immunolabelling in intestinal sections. Synaptotagmin-7 was detected in L-cells and showed similar subcellular distribution to that of GLP-1 (Fig. 1). As expected, no immunoreactivity to synaptotagmin-7 was observed in synaptotagmin-7 KO intestinal sections, where GLP-1 staining had a similar pattern to that in the control mice (Fig. 1).

Fig. 1 Detection of synaptotagmin-7 in intestinal L-cells. Intestinal sections (10 μ m) were stained with antibodies against synaptotagmin-7 and GLP-1, followed by fluorescence-conjugated secondary antibodies. Representative images, taken on a confocal microscope, are shown. Synaptotagmin-7 (green) was produced in GLP-1-positive cells and shown to have a high degree of overlap with GLP-1 signals (red). Arrows indicate selected overlapping signals of synaptotagmin-7 and GLP-1. For comparison, no immunoreactivity to synaptotagmin-7 was detected in intestinal sections from synaptotagmin-7 KO (*Syt7*^{-/-}) mice. *n*=4 mice of each genotype. Scale bars: 5 μ m (Control) and 20 μ m (*Syt7*^{-/-})

Impaired GLP-1 secretion in synaptotagmin-7 KO mice As synaptotagmin-7 functions as a calcium sensor regulating insulin and glucagon granule exocytosis, and possibly as a key regulator of other hormone and neuropeptide secretion, we tested whether deletion of synaptotagmin-7 affected GLP-1 levels and/or GLP-1 secretory response. Fasting plasma levels of GLP-1 were lower in synaptotagmin-7 KO mice than in the wild-type control (1.98 ± 0.40 vs 4.14 ± 0.53 pmol/l, $p < 0.005$), an initial indication that GLP-1 secretion is defective in synaptotagmin-7 KO mice. We then measured glucose-induced GLP-1 secretion in synaptotagmin-7 KO and control mice. Glucose ingestion induced an approximately two- to threefold increase in GLP-1 secretion from basal resting level. GLP-1 secretion increased gradually and peaked at 30 min after stimulation in synaptotagmin-7 KO and control mice. However, synaptotagmin-7 KO mice exhibited markedly reduced GLP-1 levels compared with control mice (Fig. 2a). Total GLP-1 release in response to glucose ingestion, calculated as AUC after baseline subtraction, was decreased by ~50% in synaptotagmin-7 KO mice when compared with the control mice (Fig. 2b).

Synaptotagmin-7 production in GLP-1-secreting GLUTag cells As L-cells are scattered in intestinal mucosa, it is

difficult to obtain a large number of L-cells in sufficient purity for in vitro secretion analysis. Therefore we decided to test the effects of synaptotagmin-7 deletion in a well-established murine GLP-1-secreting cell line, GLUTag cells

Fig. 2 Impaired GLP-1 secretion in synaptotagmin-7 KO mice. **a** Plasma GLP-1 levels before and 15, 30 and 60 min after glucose ingestion were determined in overnight-fasted synaptotagmin-7 KO (black circles) and control (white circles) mice. Both groups showed a similar pattern of GLP-1 secretion, but synaptotagmin-7 KO mice had much reduced GLP-1 levels at all time points. **b** Total GLP-1 secretion in synaptotagmin-7 KO (*Syt7*^{-/-}) (black bar) and control (Ctrl.; white bar) mice was calculated as AUC from the data in part **a** after baseline subtraction. *n*=12 for synaptotagmin-7 KO, *n*=15 for control mice; † $p < 0.005$

[24]. As in intestinal L-cells, synaptotagmin-7 was detected in GLUTag cells and showed a high degree of colocalisation with GLP-1 (Fig. 3).

Impaired GLP-1 secretion in synaptotagmin-7 KD GLUTag cells Due to alternative splicing at the linker region, synaptotagmin-7 is expressed in multiple variants [25]. We performed western blot analysis and detected multiple splicing forms between ~40 and ~75 kDa in GLUTag cells (Fig. 4a). To test the function of synaptotagmin-7 in GLUTag cells, we knocked down synaptotagmin-7 expression by lentivirus-mediated RNA interference (Fig. 4a). Expression of all the variants was affected by RNA interference, with synaptotagmin-7 expression reduced by more than 70% in KD cells, when compared with GLUTag cells infected with control lentivirus carrying a scrambled sequence. Other proteins, such as soluble NSF-attachment protein receptors, were not affected in synaptotagmin-7 KD cells (electronic supplementary material [ESM] Fig. 1).

Glucose-stimulated GLP-1 secretion was reduced by ~60% in synaptotagmin-7 KD cells when compared with control GLUTag cells (Fig. 4b). Using the whole-cell patch clamp technique, we also measured depolarisation-evoked GLP-1 granule exocytosis and found impaired GLP-1 granule exocytosis in synaptotagmin-7 KD cells, as indicated by the smaller capacitance jump after the depolarisation pulse (Fig. 4c). As summarised in Fig. 4d, the mean amplitude of the capacitance jump in synaptotagmin-7 KD GLUTag cells was approximately half of that in control cells. Together, these results show that GLP-1 granule exocytosis was severely impaired in synaptotagmin-7 KD GLUTag cells.

Normal GLP-1 content and calcium responses in synaptotagmin-7 KD GLUTag cells To determine whether the reduction in GLP-1 secretion and impaired GLP-1 granule exocytosis was due to defects at steps upstream of calcium sensing and membrane fusion, we first tested the

expression of proglucagon gene in synaptotagmin-7 KD and control GLUTag cells. We found no difference between the two groups (relative expression levels 1.0 ± 0.1 and 0.8 ± 0.1 for control and synaptotagmin-7 KD cells, respectively; $n=3$ independent experiments, each performed in triplicate). We also measured total GLP-1 content, finding it to be the same in control and synaptotagmin-7 KD GLUTag cells (3.0 ± 0.2 vs 2.7 ± 0.2 $\mu\text{g}/\text{mg}$ for control and synaptotagmin-7 KD cells, respectively; $n=3$ independent experiments, each performed in triplicate). We next recorded cytoplasmic calcium changes in response to 20 mmol/l glucose stimulation. High glucose induced a rapid increase in calcium levels in synaptotagmin-7 KD and control cells. There was no difference in glucose-induced calcium response pattern (ESM Fig. 2a), lag time (52 ± 3 and 50 ± 3 s, $n=15$ and 21 for control and synaptotagmin-7 KD cells, respectively [ESM Fig. 2b]) or peak amplitude of the response (267 ± 17 and 245 ± 22 nmol/l, $n=15$ and 21 for control and synaptotagmin-7 KD cells, respectively [ESM Fig. 2c]). Together, these results indicate that the impairment of GLP-1 secretion and GLP-1 granule exocytosis was due to defects downstream of the calcium signal.

Discussion

Synaptotagmin-7 functions as a calcium sensor regulating two of the key islet hormones, namely insulin and glucagon, as well as catecholamine from adrenal chromaffin cells [8, 15–17]. Like synaptotagmin-1, -2 and -9, which function as principal calcium sensors for neurotransmitter release in respective brain regions, synaptotagmin-7 appears to be a key calcium-sensing protein in endocrine and neuropeptide secretion [12–14]. In this study, we found that synaptotagmin-7 regulates GLP-1 secretion and may serve as a calcium sensor in intestinal L-cells. Synaptotagmin-7 was detected in GLP-1-positive cells in mouse intestinal epithelium. Moreover, mice with deletion of synaptotagmin-7

Fig. 3 Synaptotagmin-7 expression in GLUTag cells. GLUTag cells were stained with antibodies against synaptotagmin-7 and GLP-1, followed by fluorescence-conjugated secondary antibodies. Images were taken on a confocal microscope. Synaptotagmin-7 (green) was

present in GLUTag cells, with a high degree of overlap with GLP-1 signals (red). $n=8$ coverslips from three separate cell preparations. Scale bar: 20 μm

Fig. 4 Reduced GLP-1 granule exocytosis in GLUTag cells with lentiviral-mediated synaptotagmin-7 knockdown. **a** Immunoblot showing efficient silencing of synaptotagmin-7 expression by lentiviral-mediated RNA interference. Synaptotagmin-7 was produced in multiple isoforms in GLUTag cells. Production of all the isoforms could be efficiently knocked down. β-Actin was used as loading control. Asterisks indicate a non-specific band recognised by the synaptotagmin-7 antibody. N.I., non-infected; Sc., control infected with lentivirus carrying a scrambled sequence. **b** Reduced GLP-1 secretion in GLUTag cells with synaptotagmin-7 KD. Control (Ctrl) and synaptotagmin-7 KD GLUTag cells were incubated at 0 (basal) and then at 20 mmol/l glucose (stimulated) for 30 min. Stimulated

secretion is expressed as percentage over basal secretion. Data are presented as means±SEM of three independent experiments, each performed in triplicate. * $p<0.05$. **c** Membrane capacitance was recorded in control and synaptotagmin-7 KD GLUTag cells. Capacitance increase (fF) elicited by 500 ms depolarisations from -70 to 0 mV in synaptotagmin-7 KD cells (red trace) was lower than in control cells (black trace). Membrane conductance (pS) during the recording is also shown. **d** Recordings from similar experiments to those above (**c**), summarised and presented as means±SEM. $n=5$ and $n=7$ for control and synaptotagmin-7 KD cells; * $p<0.05$. C_m, membrane capacitance

exhibited significantly reduced GLP-1 secretion in response to oral glucose challenge. When synaptotagmin-7 expression was knocked down in GLUTag cells, high glucose-stimulated GLP-1 secretion as analysed by biochemical methods and depolarisation-evoked GLP-1 granule exocytosis as detected by membrane capacitance measurements were both reduced by more than 50% compared with control cells. KD of synaptotagmin-7 had no effect in glucose-induced calcium response, indicating that the defect in GLP-1 secretion lies at a step downstream of the calcium signal. These results are consistent with the proposal that synaptotagmin-7 plays a role as calcium sensor in L-cells, further supporting the notion that synaptotagmin-7 functions broadly as a calcium sensor in endocrine and neuroendocrine secretion.

Deletion of synaptotagmin-7 affects hormone secretion to a different extent in different cells. Whereas glucagon secretion was nearly abolished in the absence of synaptotagmin-7, insulin and catecholamine release was only reduced by 40–50% [15–17]. Although synaptotagmin-7 appears to be partially responsible for calcium-dependent GLP-1 secretion, it is difficult to determine the extent of the regulation by synaptotagmin-7 in vivo and in isolated primary L-cells without an L-cell-specific synaptotagmin-7 KO mouse line and the means of identifying L-cells in isolated primary L-cell cultures. One strategy could be to cross synaptotagmin-7 KO mouse to a previously described mouse line with fluorescence-labelled L-cells [7], and to perform electrophysiology on isolated L-cells to evaluate the extent of exocytosis defects. However, technical hurdles remain in the

process of establishing cell cultures that mimic the polarity of L-cells in intestinal epithelium [3, 7].

Since the discovery of GLP-1 in the 1980s, increasing efforts have been directed at understanding GLP-1 action and pharmacokinetics, as well as at developing therapeutic use of GLP-1-based strategies. Examples of these efforts are long half-life GLP-1 analogues and agents that inhibit GLP-1 degrading enzymes to extend the availability of endogenous GLP-1 [1, 2, 4]. Given the many benefits and success of GLP-1-based therapies in treating diabetes, it is highly relevant to understand the secretion control of GLP-1, including the biogenesis and exocytosis mechanisms of GLP-1-containing granules. While data on the mechanisms of GLP-1 secretion are sketchy, it appears that the stimulus–secretion cascade of glucose-induced GLP-1 secretion may be similar to that of pancreatic beta cells, although GLP-1 release displays a more sustained pattern compared with the biphasic pattern of insulin [3]. Considering that synaptotagmin-7 controls the final steps of GLP-1 granule exocytosis, enhancement of synaptotagmin-7 activity in triggering GLP-1 release could be a way towards fully utilising the endogenous production and secretion potential of GLP-1.

In addition to its actions on glucose sensors and food intake, GLP-1 participates in control of glycaemia by potentially stimulating insulin secretion and inhibiting glucagon secretion. We have previously shown impaired insulin secretion and nearly abolished glucagon secretion in synaptotagmin-7 KO mice [15, 16]. It is possible that reduced GLP-1 secretion could contribute to the insulin

secretion defect in synaptotagmin-7 KO mice, but the extent of the GLP-1 effect is likely to be limited, as these KO mice maintain postprandial normoglycaemia [15]. GLP-1 may inhibit glucagon secretion by acting directly on alpha cells via GLP-1 receptors on alpha cells or indirectly by stimulating insulin and somatostatin secretion [1]. In either pathway, reduced GLP-1 levels would lead to increased glucagon secretion. Thus reduced GLP-1 secretion is unlikely to have contributed to the observed glucagon secretion phenotype in synaptotagmin-7 KO mice.

In conclusion, our finding that synaptotagmin-7 serves as a positive regulator in GLP-1 secretion represents an important step in understanding the control of GLP-1 release. The present study, along with previous studies on the role of synaptotagmin-7 in islet alpha and beta cells, and in adrenal chromaffin cells, suggests that synaptotagmin-7 acts as a key calcium sensor in endocrine and neuroendocrine secretion [15–17].

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

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