Comprehensive alpha, beta and delta cell transcriptomes reveal that ghrelin selectively activates delta cells and promotes somatostatin release from pancreatic islets

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ABSTRACT

Objective: Complex local crosstalk amongst endocrine cells within the islet ensures tight coordination of their endocrine output. This is illustrated by the recent demonstration that the negative feedback control by delta cells within pancreatic islets determines the homeostatic set-point for plasma glucose during mouse postnatal development. However, the close association of islet endocrine cells that facilitates paracrine crosstalk also complicates the distinction between effects mediated directly on beta cells from indirect effects mediated via local intermediates, such as somatostatin from delta cells.

Methods: To resolve this problem, we generated reporter mice that allow collection of pure pancreatic delta cells along with alpha and beta cells from the same islets and generated comprehensive transcriptomes for each islet endocrine cell type. These transcriptomes afford an unparalleled view of the receptors expressed by delta, alpha and beta cells, and allow the prediction of which signal targets which endocrine cell type with great accuracy.

Results: From these transcriptomes, we discovered that the ghrelin receptor is expressed exclusively by delta cells within the islet, which was confirmed by fluorescent in situ hybridization and qPCR. Indeed, ghrelin increases intracellular calcium in delta cells in intact mouse islets, measured by GCaMP6 and robustly potentiates glucose-stimulated somatostatin secretion on mouse and human islets in both static and perfusion assays. In contrast, des-acyl-ghrelin at the same dose had no effect on somatostatin secretion and did not block the actions of ghrelin.

Conclusions: These results offer a straightforward explanation for the well-known insulinostatic actions of ghrelin. Rather than engaging beta cells directly, ghrelin engages delta cells to promote local inhibitory feedback that attenuates insulin release. These findings illustrate the power of our approach to resolve some of the long-standing conundrums with regard to the rich feedback that occurs within the islet that is integral to islet physiology and therefore highly relevant to diabetes.

Keywords Ghrelin; Delta cell; Somatostatin release; Transcriptome; Beta cell; Alpha cell

1. INTRODUCTION

Pancreatic alpha and beta cells co-localize in an arrangement that facilitates accurate coordination of glucagon and insulin release from islets. While glucagon and insulin, along with Islet amyloid polypeptide (lapp), are among the few islet signals that meet the classic definition of a hormone as a ‘factor that is released into the general circulation to signal at a distant site in the body’, a rich constellation of paracrine and neural interactions takes place within the islet to ensure tight control over their release [1–3]. Pancreatic delta cells are the third-most common endocrine cell type in the islets, and the somatostatin they release is an important inhibitor of both insulin and glucagon [4–6]. We recently described a novel negative feedback loop where the paracrine peptide Urocortin 3 (Ucn3) is co-released with insulin from beta cells and promotes glucose-stimulated somatostatin release via the type 2 corticotropin-releasing hormone (Crhr2) receptor expressed...
by delta cells [7]. Somatostatin then attenuates further insulin secretion and helps to maintain stable and tight control over plasma glucose. The appearance of Ucn3 across the beta cell mass correlates with the well-known uptick in plasma glucose levels [8–10]. The onset of Ucn3 is directly responsible for this phenomenon by initiating delta cell-dependent feedback on insulin release, thus determining the homeostatic set-point for plasma glucose [7]. Delta cell-mediated feedback breaks down early in diabetes, which leads to marked increases in plasma glucose fluctuations that resemble the glycemic volatility that impacts patients across the diabetes spectrum [7]. While these observations highlight the physiological importance of delta cell-dependent feedback, we know relatively little about the cues that control delta cells. The intimate co-localization of islet endocrine cells has also made it difficult to distinguish direct effects on beta cells from indirect actions that are mediated by locally produced intermediaries. We previously generated a transgenic mIns1-H2b-mCherry reporter mouse in which beta cells are labeled by the nuclear expression of mCherry driven by the mIns1 promoter and crossed these to S100b-eGFP reporter mice that we serendipitously discovered to label alpha cells [11]. These reporter mice enabled the comparison of mouse and human beta cell transcriptomes but did not fully resolve the transcriptomes of pancreatic alpha and delta cells. To improve substantially on these prior observations and to determine the factors that directly engage delta cells, we now generated a set of triple transgenic mouse models in which mIns1-H2b-mCherry [11] beta cells are crossed to mice with alpha or delta cells are marked by YFP in a Cre-dependent fashion. We used these reporter mice to generate unbiased and comprehensive transcriptomes of pancreatic delta and alpha cells, along with beta cells from the same islets. Our transcriptomes are validated by the strong enrichment for a large panel of known alpha, beta, and delta cell markers in the appropriate cell type. We then use this information to determine the signals that directly engage the delta cell by virtue of selectively expressed receptors and analyze ghrelin stimulation of delta cells as an example to highlight the utility of our approach. We discovered that ghrelin acts directly on mouse and human delta cells to promote somatostatin release. Our observations give a straightforward explanation for the well-known insulinostatic actions of ghrelin (e.g. [12–16]) and illustrate the physiological importance of delta cell-mediated feedback within pancreatic islets. An accurate understanding of ghrelin’s mechanism of action within pancreatic islets is highly relevant given ghrelin’s central role in the regulation of energy and glucose metabolism [17].

2. RESEARCH DESIGN AND METHODS

2.1. Biological materials and ethics statements

All mouse procedures were approved by the UC Davis or the Salk Institute for Biological Studies Institutional Animals Care and Use Committee and were performed in compliance with the Animal Welfare Act and the Institute for Laboratory Animal Research (ILAR) Guide to the Care and Use of Laboratory Animals. Animals were maintained on a 12-h light/12-h dark cycle with free access to water and standard rodent chow. Static and dynamic hormone secretion experiments were carried out on C57BL/6Nhsd mice, between 8 and 16 weeks of age, from Harlan (Indianapolis, IN). We obtained human islets via the Integrated Islet Distribution Program; the receipt was declared exempt from Harlan (Indianapolis, IN). We obtained human islets via the Integrated Islet Distribution Program; the receipt was declared exempt from Harlan (Indianapolis, IN). We obtained human islets via the

2.2. Immunofluorescence and FISH

Immunofluorescence was conducted as previously described [7,8]. Insulin was detected using guinea pig anti-insulin (Dako #A0564; 1:500), somatostatin using sheep anti-somatostatin (American Research Products Inc. #13-2366, 1:1000), Ucn3 using rabbit anti-Ucn3 (#6570; in house, 1:2000), Glucagon using rabbit anti-glucagon (Abcam #ab11022-1; 1:200), and YFP using goat anti-GFP (Rockland 600-101-215; 1:1000). All secondary antibodies were obtained from Jackson Laboratories Inc. RNA FISH was conducted using RNAscope probes for Ghsr and Sst (Advanced Cell Diagnostics) according to the manufacturers instructions.

2.3. Islet isolation and FAC sorting

Islet isolation was conducted as previously described [7,11,18]. Islets from mIns1-H2b-mCherry [11] (deposited with the Jackson laboratories as strain #28589) × Rosa-LSL-YFP [19] × Sst-Cre [20] or Gcg-Cre [21] triple transgenic animals were pooled by sex in 2 (Sst-Cre) or 3 (Gcg-Cre) replicate groups of a dozen animals. FACs sorting was conducted as described previously [7,11] with each sample collected directly in Trizol to ensure immediate cell lysis and preservation of RNA integrity.

2.4. Next generation sequencing and bioinformatics

RNA was isolated from Trizol-preserved samples by chloroform extraction and cleaned up over an RNeasy microcolumn essentially as previously described [11]. RNA quality was verified by Tapestation (Agilent, Santa Clara, CA). Indexed sequencing libraries were constructed using the TrueSeq RNA sample Prep Kit v2 (Illumina Inc. San Diego, CA), sequenced at 50 cycles, and single read on an Illumina HiSeq 2000 platform. Results were validated by qPCR using Sybr chemistry and the primers listed in Table 1. Sequencing reads were mapped to the mouse genome version GenCode M5 (GRCm38.p3) using STAR v2.4 [22]. On average over 33 million reads were sequenced for each library with 89.9% of sequenced reads aligning (>63% unique alignment overall). FeatureCounts [23] was used to create count tables of the sorted bam files using reads aligning to RefSeq-defined exons. EdgeR version 3.12.0 [24] was used to conduct pairwise statistical comparisons. Wordles of transcript abundance were generated on wordle.net. Single cell RNAseq data from [25] were used to generate the violin plots in Figure 2C. Cells that had an RPKM value > 10 k of either Sst, or Ins2, or Ggc were defined as delta, beta, or alpha cells, respectively.

2.5. Functional imaging by GCaMP6

Sst-Cre mice and LSL-GCaMP6 mice (Jackson laboratories strain #24106) were crossed for functional imaging. Intact islets from bitransgenic offspring were plated on poly-D lysine-coated number 1.5 35 mm glass cover slip tissue culture dishes (Mattek) and maintained at 37 °C 5% CO2 in RPMI 10% FBS, 5.5 mM glucose with pen/strep.

Table 1 — qPCR primer information.

| Ref Seq ID | Gene | Primer | Sequence 5’ -> 3’ | Amplification size (bp) |
|------------|------|--------|-------------------|-------------------------|
| NM_008100  | Ggc  | grodGgc.fwu1 grodGgc.rvu1 | TCACAGGGCAATTCACAGCATTTGCAAGACGAGTTC | 121 |
| NM_00185084| Ins2 | grodIns2.fwu1 grodIns2.rvu1 | GCTCTCTGAGCTGTGGTGCGTTGTTGACG | 128 |
| NM_009215  | Sst  | grodSst.fw grodSst.rv | GACCCCAAGCTCCGTAATTTCGGTGGTGGTGG | 112 |
| NM_021488  | Ghse | qmGhse.fwu1 qmGhse.rvu1 | TCTCTGCTGTGGTGGTGGTGGG | 113 |
| NM_0153556 | Hprt| qmHprt.fwu qmHprt.rvu | TTCCTGCTGAAGCTCAGCAAGA | 90 |
Islets were allowed to adhere for 24 h prior to imaging using a Nikon A1R confocal microscope. During live cell fluorescence imaging acquisition, islets were continuously superfused with 37 °C Krebs bubbled with 95% O₂ and 5% CO₂. To measure baseline, high glucose, glucose plus ghrelin, and control responses, buffers were switched at the indicated time points. The position of delta cells within the islet in X, Y, and Z were first identified during a brief pulse with 16.8 mM glucose. Afterwards, islets were washed with 2.8 mM glucose for 30 min to allow fluorescence to return to baseline. For fluorescence intensity analysis, GCAmp6 glucose responsive regions of interest corresponding to single delta cells were identified and defined during the maximal depolarization by 30 mM KCl. Data analysis was performed using Nikon Elements software.

2.6. Hormone secretion

Secretion experiments were conducted in Krebs—Ringer Buffer (KRB). We hand-picked the required number of islets from a pool of all islets and assigned these as the next replicate to each subsequent treatment. Static somatostatin secretion was carried out using 50 or 100 islets per well for mouse islets under high or low glucose, respectively. 100 islets/well were used for human islet release. Dynamic somatostatin release was determined using a custom-built perfusion setup using 200 islets in 6 parallel chambers. We measured somatostatin by RIA [26] using anti-Sst14 antiserum (S201; diluted 1:50,000) [27] as described [7]. EC50 and minimal detectable dose are 12 pg/tube and 1 pg/tube, respectively. Insulin was measured by commercial RIA (Millipore) as previously described [18]. Ghrelin, des-acyl-ghrelin, Astressin2b, and the Sstr3 antagonist Sst3—ODN—8 (Carbamoyl—des—A1,2,4,5,12,13[p—Cys3,Tyr1,p—AgI8] (Me,2—naphthy]—SS; #315—260—15) [28] were generously synthesized and provided by Drs. Michael Beyermann and Jean Rivier.

2.7. Statistical analyses

Statistical analyses were carried out in Prism 6.0e for Mac (GraphPad Software, Inc.). We reported all values as mean values across bio-logical replicates and assumed normality, unless otherwise noted. We evaluated experiments with more than two treatments by ANOVA followed by Student’s t-test to determine which means differed statistically, and we applied Welch’s correction for unequal variance when necessary. We evaluated perfusion data by two-way ANOVA for treatment and the interaction of treatment and time for each block.

3. RESULTS

3.1. Generation and validation of alpha, beta and delta cell transcriptomes

To obtain transcriptomes of mouse alpha, beta, and delta cells, we crossed our mice into H2b-mCherry mice [11] to either Sst-Cre [20] or Gcg-Cre [21] mice and a Rosa-LSL-YFP reporter [19] (Figure 1A—D). The resulting triple transgenic offspring enabled FACS purification of highly pure beta cells and delta or alpha cells that we used to generate comprehensive transcriptomes of mouse delta, beta, and alpha cells. Beta cell transcriptomes were markedly enriched for mCherry expression (Figure 1E), while alpha and delta cell transcriptomes were each marked by strong enrichment for YFP (Figure 1F), underpinning our robust FACS-based purification. Alpha, beta, and delta cells are all endocrine cells most noted for the release of glucagon, insulin, and somatostatin, respectively. Indeed, somatostatin transcripts are by far the most abundant transcripts in delta cells and account for 3.4 ± 0.25% of all aligned reads in delta cells (Figure 1G). Insulin (encoded by Ins2 and Ins1 genes in mouse) makes up 18.9 ± 2.4% of all reads in the beta cell transcriptome (Figure 1H), while glucagon transcripts make up 27.8 ± 0.9% of all reads in alpha cells (Figure 1I). Lapp is the second-most abundant transcript in our delta cell transcriptomes and while lapp is best-known as a beta cell hormone, delta cells also express lapp [29].

3.2. Transcriptome validation

We observed on average 1369 genes that were differentially enriched in each pairwise comparison, as defined by a Log2-fold expression > 1 or < −1 and a FDR < 0.00001. Known delta cell markers such as Sst, Rbp4, and Hhex [30,31] were highly enriched in pairwise comparisons to beta and alpha cells (Figure 1J,K). Known beta cell markers such as Ucn3, Mafa, Ins1, Ins2, and Nkx6-1 [11] were markedly enriched in beta compared to delta and alpha cells (Figure 1J,L). Alpha cell markers such as Ggc, Tr, Inx1, Inx2, Arx, and Mab2 [30,32,33] were enriched in alpha cells compared to delta and beta cells (Figure 1K,L). As Pdx1 is expressed in beta and delta cells (Figure 1M), it was enriched in neither cell type upon pairwise comparison (Figure 1J).

3.3. Islet cell transcriptomes reveal GPCR expression profiles

Given the importance of paracrine interactions to control islet insulin and glucagon output [7], we assessed the expression of GPCRs in our islet transcriptomes in more detail. As noted, Ctr2 is expressed exclusively by delta cells, but several GPCRs that are well-known for their beta cell expression are also expressed to a lesser extent by delta cells but absent from alpha cells (Figure 2A). This group includes glucagon-like peptide 1 receptor (Glpt1), the alpha 2 adrenergic receptor (Adra2a), and the glucagon receptor (Ggc2). In contrast, the gastric inhibitory polypeptide receptor (Gipr) is ubiquitously expressed in the islet and is detected in approximately equal measures by alpha and delta cells as well. In contrast, the ghrelin receptor 1 (Gar1) is expressed selectively in beta cells. In searching for GPCRs that, like Ctr2, are expressed selectively by delta cells, we noted the delta cell-specific expression of Sst1t. Alpha cells selectively express Sst2, while Sst3 is expressed by alpha, beta and delta cells. There is no detectable expression of Sst4 or Sst5 in any of the endocrine cells of the mouse islets. Another example of a GPCR that caught our attention as expressed selectively by delta cells is the growth hormone secretagogue receptor (Ghsr), which encodes the GHS-1R that responds to ghrelin (Figure 2A). We validated the selective expression of Ghsr in delta cells by qPCR (Figure 2B). A recently published data set of high quality, single cell RNAseq of dissociated wild type mouse islets that contains populations of Sst-expression delta cells, Ins2-expression beta cells and Ggc-expression alpha cells [25] supported these observations, as Ghsr in these data was also detectable only in delta cells (Figure 2C). We further confirmed Ghsr mRNA expression in delta cells by FISH. We found that Ghsr mRNA (green) colocalizes with Sst mRNA (red) in cells at the islet periphery that co-express Sst peptide (white) (Figure 2D) but not Ggc peptide (white) (Figure 2E).

3.4. Ghrelin directly activates calcium in delta cells

GHS-1R primarily couples through Gαq [34], which results in phospholipase C-mediated intracellular calcium release. Thus, we evaluated the calcium response of delta cells to ghrelin in intact islets. We used islets from bitransgenic Sst-Cre [20] × LSL-GCaMP6 mice that express the Ca²⁺ sensor GCaMP6 [35] specifically and selectively in delta cells. After establishing baseline fluorescence under KRB with 2.8 mM glucose, increasing glucose to 16.8 mM glucose led to a
marked increase in delta cell GCaMP6 fluorescence intensity, which gradually tapered down (Figure 3; Supplementary Video 1). Upon subsequent addition of 100 nM ghrelin delta cells responded with a large initial Ca\(^{2+}\) peak that was followed by a distinct oscillatory response (Figure 3). The Ca\(^{2+}\) signal returned to baseline upon cessation of stimulation. Ghrelin stimulation under 2.8 mM glucose produced no increases GCaMP6 signal (not shown). Depolarization by 30 mM KCl at the conclusion of the experiment resulted in a robust GCaMP6 calcium peak, confirming delta cell viability and responsiveness throughout the experiment.

3.5. Ghrelin promotes somatostatin secretion

Given the increase in intracellular calcium upon activation of GHS-1R on delta cells and the importance of calcium in hormone secretion, we proceeded to determine if ghrelin promotes somatostatin secretion. Indeed, stimulation with ghrelin strongly potentiated glucose-stimulated somatostatin secretion (GSSS) in mouse islets (Figure 4A). Des-acyl-ghrelin, which lacks the octanyl post-translation modification at the Ser3 position and does not activate GHS-1R, did not potentiate GSSS. To determine if the actions ghrelin depended on the feedback mediated by beta cell-derived Ucn3, we blocked the
actions of endogenous Ucn3 with the CRHR2-selective antagonist Astressin2b. Astressin2b attenuated GSSS as previously reported [7], but did not prevent the ghrelin-induced potentiation of GSSS (Figure 4B). This indicates that ghrelin engages delta cells directly, independent of Ucn3-mediated potentiation of GSSS [7]. Ghrelin, but not des-acyl-ghrelin, robustly potentiated GSSS from human islets in vitro, confirming that its actions are similar across rodents and primates (Figure 4C). We next tested the actions of ghrelin on somatostatin secretion in an islet perfusion setup and observed that islets demonstrated robust potentiation of GSSS in response to 100 nM ghrelin. Islets that did not receive ghrelin continued to maintain glucose-stimulated somatostatin levels (Figure 4D). Des-acyl-ghrelin did not elicit any response following a 15-minute pre-incubation and did not block the potentiation of GSSS by ghrelin (Figure 4E). Islets in all perfusion experiments responded to brief depolarization with 30 mM KCl with a strong somatostatin peak, confirming islet viability.

3.6. Ghrelin attenuates insulin release in a somatostatin-dependent manner

We next assessed whether the insulinostatic effect of ghrelin depended on intact somatostatin-mediated feedback within the islet. Stimulation of wild type mouse islets with ghrelin attenuated glucose-stimulated insulin secretion (Figure 5), in line with most published reports. Co-administration with an antagonist to the Sstr3 receptor [28], which is the only Sst receptor that is detectably expressed by primary mouse beta cells (Figure 2A), blocked the insulinostatic actions of ghrelin completely (Figure 5). These observations demonstrate that ghrelin’s insulinostatic actions depend on intact Sstr3-dependent feedback within the islet. The fact that Sstr3 blockade promoted glucose-stimulated insulin secretion suggest that this Sstr3-mediated feedback exerts a tonic inhibitory effect on glucose-stimulated somatostatin secretion.

4. DISCUSSION

The importance of the neural, endocrine, and paracrine inputs to pancreatic islets that collectively determine the output of insulin and glucagon has long been recognized. However, the tools and techniques at our disposal to map this complex local crosstalk have lacked the resolution and specificity to accurately determine the origin of many signals and the identity of the islet cell receptive to its message. The recent onset of next-generation sequencing approaches has now made it attainable to conduct an unbiased evaluation of the expression of all genes in any given tissue that is quantitative over many orders of magnitude. The purity and quality of the input sample has become the factor limiting the usefulness of the data achieved in this way. For pancreatic islets, which are often equated with beta cells, but which contain many additional endocrine and non-endocrine cells, we resolved this issue by generating triple transgenic reporter mice that collectively enable the FACS-purification of alpha, beta, and delta cells from the same islets. From these highly pure input samples, we generated comprehensive gene expression compendia of the three principal endocrine cells within the islets. This now enables us to unravel with great accuracy some of the crosstalk pathways that had to date been unknown. Our current transcriptome data are a distinct improvement over the work we published previously [11] and offer a
significantly better resolution between pancreatic alpha and delta cells. Our data sets complement a recent, high quality single cell RNAseq data set of dissociated mouse islets [25]. The single cell approach offers valuable insight into the heterogeneity of gene expression amongst the different endocrine populations in the islet and complement our transcriptomes from FACS-purified populations of alpha, beta, and delta cells that demonstrate a far greater depth and dynamic range and therefore reliably detect the expression of a far greater number of genes, as expected. While the most prevalent transcript in alpha, beta, and delta cell transcriptomes was Gcg, Ins2, and Sst, respectively, the relative abundance of Gcg in alpha cells (27.8%) and Ins2 in beta cells (18.9%) was much greater than the relative abundance of Sst in delta cells, which made up merely 3.5% of all aligned reads. As our transgenic reporter strategy is very similar for delta and alpha cells (Figure 1), there is no technical reason for delta cells to exhibit such a relatively modest gene expression of their principal endocrine signal Sst, in comparison to the expression of Gcg and Ins2 in alpha and beta cells, respectively. Instead, we think that this is a direct reflection of the fact that insulin and glucagon are hormones released into the systemic circulation to act at sites throughout the body, while somatostatin is responsible for local feedback within the islet. Our transcriptomes analyses reinforce the view that delta cells are similar to beta cells. This extends from the mechanistically similar control of glucose-stimulated exocytosis in both beta and delta cells that proceeds via K<sub>ATP</sub> channels, voltage-gated sodium, and calcium channels [7,36] to include GPCR control that can potentiate glucose-stimulated exocytosis in both cell types. Some of these GPCRs, such as Chhr2 and Ghlsr on delta cells and Galr1 on beta cells, are selectively expressed by only one cell type. In contrast, a series of other GPCRs that are routinely associated with beta cells, including Gpr1r, Adra2a, and Gcgr, are expressed by delta cells at likely physiologically meaningful levels. We do not think that these reflect contamination by beta cells, as many known beta cell-specific genes are indeed highly selectively detected in our beta cell transcriptomes only and do not demonstrate similar levels of expression by delta cells (Figure 1). These observations betray significant overlap in delta and beta cell transcriptional programs, possibly afforded in part by the shared expression of Pdx1. These similarities likely reflect the close ontogenetic origins of beta and delta cells and are in line with observations

Figure 4: Ghrelin potentiates glucose-stimulated somatostatin secretion. A: Ghrelin significantly increases somatostatin release from mouse islets in vitro. B: Ghrelin, but not des-acyl-grelin, promotes somatostatin release. Astressin2b attenuates glucose-stimulated somatostatin release by preventing beta cell-derived Ucn3 from stimulating alpha cells. C: Ghrelin, but not des-acyl-grelin, promotes glucose stimulated somatostatin secretion from human islets in vitro. D: Perfusion of mouse islets under high glucose with ghrelin (black circles) acutely potentiates glucose-stimulated somatostatin secretion compared to control (open circles). E: Application of des-acyl-ghrelin before and during stimulation does not affect ghrelin's actions are independent from the feedback mediated by Ucn3. C: Ghrelin, but not des-acyl-grelin, promotes glucose stimulated somatostatin secretion from human islets in vitro.
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attenuates glucose-stimulated insulin secretion from wild type mouse islets

ghrelin [40].

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glucagon [38].

The transcriptomes we report here constitute an invaluable repository that

informs on the expression, or lack thereof, of all genes in each of the

main endocrine cell types of the islet, including delta cells. In addition to confirming that Ctr2 is selectively expressed by delta cells

[7], we discovered that the Ghsr gene is abundantly and selectively expressed in delta cells (Figure 2). Stimulation of mouse and human

islets with ghrelin indeed leads to a robust increase in somatostatin

secretion (Figure 4) and stimulates calcium responses in delta cells in

intact islets (Figure 3). Furthermore, at equimolar concentrations, des-

acyl-ghrelin in intact islets does not affect somatostatin secretion and
does not block ghrelin-induced somatostatin release, in agreement with prior observations that des-acyl-ghrelin is a full agonist of the

GHS-R1 only at very high concentrations [39] and does not block ghrelin [40].

Most studies to date have assumed that the Ghsr within islets is expressed by beta cells and thereby inhibits insulin release [41]. This

implies that GHS-R1 in islets is not coupled to its canonical G\textsubscript{q/11}
signaling cascade [42], as this would enhance rather than attenuate

insulin secretion. Moreover, the insulinostatic effects of ghrelin are

pertussis toxin-sensitive [43], indicative of the involvement of G\textsubscript{q}. Our

findings resolve this long-standing paradox [17] by demonstrating that

ghrelin promotes somatostatin release, likely by engaging the can-
nonical G\textsubscript{q/11} and Ca\textsuperscript{2+} cascade in delta cells, which would then inhibit insulin via beta cell somatostatin receptors coupled to G\textsubscript{q}. Such a model is in good agreement with the preponderance of published data on the mechanism by which ghrelin inhibits insulin secretion, but offers a more parsimonious explanation compared to some of the prior

models that have been proposed to resolve the discrepancy between

ghrelin’s well-accepted insulinostatic actions and the fact that its

canonical signaling cascade would promote, not inhibit, exocytosis.

It has been suggested that Ghsr is expressed by alpha cells and

promotes glucagon release [44]. This was based on Ghsr detection

by in situ hybridization at the islet periphery, where alpha cells are

intimately associated with delta cells [44], experiments on the alpha-

TC-1 alpha cell line in which expression of Ghsr need not imply

similar Ghsr expression by primary alpha cells, and studies on iso-

lated islets under 12 mM glucose, a concentration not typically

associated with highest alpha cell activity. More importantly, in-

creases in glucagon stimulate insulin [45,46] and, therefore, cannot

explain the insulinostatic actions of ghrelin that are generally

accepted [12–16]. Hetero-dimerization between GHS-R1 and SST5

on the beta cell surface has been offered to explain how ghrelin

inhibits insulin release in a G\textsubscript{q/11}-dependent manner [47]. The fact that

Ghsr is expressed by delta cells and that Sstr5 is not detected in any

mouse islet endocrine cell (Figure 2) makes this model untenable.

More recently, coupling of the GHS-1R with transient receptor po-
tential melastatin 2 (TRPM2) was suggested as an alternate expla-

nation to reconcile ghrelin’s insulin inhibition with G\textsubscript{q/11}

and phospholipase C-mediated increases in Ca\textsuperscript{2+} [48]. Attenuation by

ghrelin of non-selective glucose-induced cation currents via TRPM2

that promote insulin release led to a model in which TRPM2 was

placed downstream of Ghrelin/GHS-R1 on beta cells [48]. The fact

that we demonstrate that Ghsr is expressed exclusively by delta not

by beta cells would not alter the hierarchy of Trmp2 downstream of

ghrelin/GHS-1R but would merely insert somatostatin as an inter-

mediate factor between ghrelin and Trpm2.

Our observation that beta cells do not express Ghsr is in agreement

with a prior report that detected no Ghsr on HIT-T15 beta cells [49].

This study observed that ghrelin promotes beta cell proliferation and

prevents beta cell apoptosis. Since these effects were also observed in

response to des-acyl-ghrelin and depended on adenyl cyclase and

protein kinase A, it was suggested that they are mediated via an as yet

unidentified receptor distinct from GHS-R1 [49]. This is certainly an

intriguing possibility, and we do not rule out the presence of an

alternate receptor with high affinity for ghrelin and des-acyl-ghrelin in

the islet. If an alternate ghrelin receptor indeed exists within the islet,

this suggests the possibility of secondary effects of ghrelin stimulation

that are mediated indirectly via local intermediates. Nevertheless, we

report here that the actions of ghrelin (and not des-acyl-ghrelin) are in

full agreement with the selective activation of delta cell GHS-R1 by

ghrelin. There has been a prior report that suggested ghrelin had no

effect on basal somatostatin release but modestly inhibited arginine-

induced somatostatin secretion in perfused rat pancreas [50]. We

are unable to offer an explanation that reconciles this observation with

our current data.

We think that many of the discrepancies with regard to the mechanistic

understanding of ghrelin’s insulinostatic actions can be traced to ob-

servations that place Ghsr expression on alpha or beta cell lines. As the

canonical signaling cascade in response to ghrelin would promote

exocytosis, stimulation of insulin (from beta cell lines) or glucagon

(from alpha cell lines) in response to ghrelin is the default response.

However, caution should be exercised when extrapolating these ob-

servations on cell lines to the primary endocrine cells they resemble

most closely. All islet endocrine cells derive from a common lineage.

Key characteristics of primary islet endocrine cells are often lost in cell

lines, with traits normally restricted to one particular cell type reap-

pearing in cell lines that are thought to resemble another islet endo-

crine cell. The transcriptomes we report here constitute a valuable
repository to validate the cell type that expresses any gene that is
detectably expressed within pancreatic islets.

5. CONCLUSIONS

We contribute significantly to the understanding of the physiological
role of ghrelin by offering an alternate mechanistic explanation how
ghrelin achieves its well-known insulinostatic actions, which is rele-
vant given ghrelin’s central role in metabolism [17]. Moreover, our
observations underscore that delta cells play an underappreciated but
important physiological role in controlling insulin output by local
feedback within pancreatic islets [7]. The high quality transcriptomes
from delta, beta, and alpha cells that were instrumental in this dis-
covery will be invaluable to unravel the complex crosstalk that tightly
balances insulin and glucagon release from healthy islets and breaks
down in diabetes.

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AUTHOR CONTRIBUTIONS

MRD conducted functional imaging on intact islets and contributed to
writing and discussions. AM and CC-Z conducted all bioinformatics
analyses and contributed to discussions. CJD and JV measured so-
matostatin secretion, generated tracers and and maintained the RIA for
somatostatin. GN conducted immunofluorescence and FISH experi-
ments. TvdM conducted experiments and contributed to the writing,
editing and discussions. MOH designed the study and directed the
project, generated delta, beta, and alpha cell transcriptomes, con-
ducted static and dynamic somatostatin secretion experiments and
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CONFLICT OF INTEREST

The authors have no conflicts to declare.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.
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