CellPress

Cell Metabolism
Resource

High-Throughput Luminescent Reporter of Insulin Secretion for Discovering Regulators of Pancreatic Beta-Cell Function

Sean M. Burns,[1,2,3,6,*] Amedeo Vetere,[7] Deepika Walpita,[7] Vlado Dančík,[7] Carol Khodier,[8] Jose Perez,[8] Paul A. Clemons,[7] Bridget K. Wagner,[7] and David Altshuler[1,2,3,4,5,6]
[1]Diabetes Unit of the Department of Medicine
[2]Center for Human Genetic Research
[3]Department of Molecular Biology
Massachusetts General Hospital, Boston, MA 02114, USA
[4]Department of Genetics, Harvard Medical School, Boston, MA 02115, USA
[5]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02142, USA
[6]Medical and Population Genetics Program
[7]Center for the Science of Therapeutics
[8]Center for the Development of Therapeutics
Broad Institute of Harvard and MIT, Cambridge, MA 02142, USA
*Correspondence: sburns@broadinstitute.org
http://dx.doi.org/10.1016/j.cmet.2014.12.010

SUMMARY

Defects in insulin secretion play a central role in the pathogenesis of type 2 diabetes, yet the mechanisms driving beta-cell dysfunction remain poorly understood, and therapies to preserve glucose-dependent insulin release are inadequate. We report a luminescent insulin secretion assay that enables large-scale investigations of beta-cell function, created by inserting *Gaussia* luciferase into the C-peptide portion of proinsulin. Beta-cell lines expressing this construct cosecrete luciferase and insulin in close correlation, under both standard conditions or when stressed by cytokines, fatty acids, or ER toxins. We adapted the reporter for high-throughput assays and performed a 1,600-compound pilot screen, which identified several classes of drugs inhibiting secretion, as well as glucose-potentiated secretagogues that were confirmed to have activity in primary human islets. Requiring 40-fold less time and expense than the traditional ELISA, this assay may accelerate the identification of pathways governing insulin secretion and compounds that safely augment beta-cell function in diabetes.

INTRODUCTION

Type 2 diabetes (T2D) represents a global health epidemic that is predicted to double in prevalence over the next 20 years, causing blindness, amputation, neuropathy, and life-threatening macrovascular complications (Boyle et al., 2010). The disease is characterized by impaired insulin action in target organs and a failure of insulin secretion to compensate for this defect. Progression of disease correlates closely with decreases in insulin secretion (Fonseca, 2009), and human genetic studies of both Mendelian and common forms of diabetes have highlighted a central role of beta-cell dysfunction in the inherited basis of T2D (Ingelsson et al., 2010). Identifying the mechanisms by which beta cells fail in diabetes, and targeting such pathways therapeutically, could prevent the development of the condition and significantly improve its treatment.

Historically, assays of beta-cell function have relied on the ELISA, the gold standard for measuring insulin. This method, while of critical importance to the field, is characterized by multiple liquid-handling steps, limited dynamic range, and high cost (> $2 per sample), making it challenging for use in high-throughput applications. Recently, 384-well insulin assays based on fluorescence resonance energy transfer (FRET) have become commercially available (Degorce, 2006; Bielefeld-Sevigny, 2009). While these assays enable larger format experiments, they remain relatively expensive ($0.60–$1.70 per sample), require supernatant transfer steps, and involve plate readers with advanced hardware compatible with these methods (i.e., time-resolved FRET; AlphaLISA laser-based detection). As a consequence, only two small-scale chemical screens of insulin secretion have been published to date, and the size of each (1,280 and 4,691 compounds) limited their ability to detect novel insulinotropic compounds (Wu et al., 2008; Lee et al., 2011).

Several groups have attempted to circumvent the ELISA using surrogate markers of insulin secretion, by fusing either GFP or luciferase to the end of the insulin propeptide (Pouli et al., 1998; Liu et al., 2007; Suzuki et al., 2011). While some have been useful for tracking exocytotic events, none have demonstrated correlation with the insulin ELISA. Moreover, the constructs appear to have detrimental effects on cell function, possibly due to protein misfolding. Over 95% of a proinsulin-firefly luciferase fusion protein is retained in the ER, and cells expressing a proinsulin-*Gaussia* luciferase construct show less than 3-fold induction of secretion upon maximal glucose stimulation.

A rapid and inexpensive way of tracking insulin secretion would enable the systematic and comprehensive evaluation of

genetic and environmental factors contributing to insulin deficiency in diabetes and the search for compounds reversing these defects. Here, we report the design and validation of a luminescent insulin secretion assay created by inserting *Gaussia* luciferase within the C-peptide portion of the insulin prohormone, flanked by additional prohormone convertase cleavage sites. Upon stimulation with known insulin secretagogues, beta cells expressing this reporter secrete luciferase in close correlation with insulin, under standard conditions as well as upon induction of cellular stress. At a cost of $0.05 per sample, the assay requires > 40-fold less time and expense than the traditional ELISA. Moreover, it performs well in high-throughput applications, and can be used without a supernatant transfer step. Using the reporter, we performed a proof-of-principle, 1,600-compound screen, under both low- and high-glucose conditions, identifying several small molecule modulators of beta-cell secretion. Top-scoring compounds from the high-glucose screen were subsequently confirmed to augment glucose-stimulated insulin secretion (GSIS) from cadaveric islets, validating the relevance of the assay to human biology. Moreover, one of the drugs found to decrease secretion in our screen, fenofibrate, was subsequently confirmed to inhibit insulin secretion from human islets, demonstrating the potential of the assay for identifying negative effects of commonly used medications on beta-cell function.

RESULTS

Creating a Luminescent Insulin Secretion Reporter

We reasoned that luciferase could be used to track insulin secretion if properly targeted to the secretory vesicles within the beta cell via the endogenous proinsulin processing pathway, in a manner that preserved the activity of the enzyme. To accomplish this, we constructed a fusion protein in which the sequence of *Gaussia* luciferase was placed within the C-peptide portion of mouse proinsulin, a fragment that is normally cleaved within the vesicles by pH-sensitive prohormone convertase enzymes and cosecreted with mature insulin during exocytosis (Oyer et al., 1971). *Gaussia* luciferase was chosen because it is the smallest and brightest luciferase identified to date (Tannous et al., 2005). To ensure full activity of the luciferase, a version was used that was codon-optimized for expression in mammalian cells, and we flanked the enzyme with additional cleavage sites for prohormone convertase 2 (PC2), such that no extra amino acids would be present on the ends of the enzyme after processing within the vesicles (Figure 1A).

In the absence of a suitable glucose-responsive human beta-cell line, we created both mouse (MIN6; Miyazaki et al., 1990) and rat (INS-1E; Merglen et al., 2004) beta-cell lines stably expressing the reporter construct. Each line was indistinguishable from its wild-type counterpart in terms of morphology, replication rate, and viability. The luminescent reporter of insulin secretion was validated in both cell lines, with highly similar results between species.

Upon challenging INS-1E cells expressing the reporter with varying concentrations of glucose for 1 hr, secretion of insulin and luciferase were highly correlated ($r^2 = 0.99$; Figures 1B and 1C). Similar results were obtained from MIN6 cells (Figure S1 available online). To confirm that the proinsulin-luciferase fusion protein tracked with insulin and was not retained in the ER, immunohistochemistry was performed on single cells. Confocal imaging revealed nearly complete colocalization of insulin and luciferase within the secretory granules, suggesting that the fusion protein folds properly and enters the natural proinsulin-processing pathway (Figures 1D, 1E, and S2). Next, to determine if the reporter could be used to track insulin secretion from human beta cells, dissociated human islets were transduced with a lentiviral expression vector containing the reporter. Upon stimulation of these cells with varying glucose levels, luciferase and insulin were secreted in close correlation (Figure 1F). The effect of the fusion construct on beta-cell function and endogenous insulin secretion was then evaluated by comparing the C-peptide response of cells with and without the transgene. No significant difference was found upon stimulation with glucose, suggesting that, unlike other reported fusion proteins, our reporter does not impair normal insulin processing (Figure 1G).

We further tested the time course of glucose-stimulated secretion in the INS-1E cell line, observing a predominantly monophasic pattern, with the magnitude of secretion increased by the known glucose-dependent insulin secretagogue 3-isobutyl-1-methylxanthine (IBMX; Figure 1H). To confirm that cells expressing the reporter secrete luciferase via the regulated insulin secretion pathway, we examined the effect of diazoxide, a compound known to hyperpolarize the beta cell and inhibit insulin secretion. As expected, the compound also inhibited luciferase secretion from cells expressing the fusion protein (Figure 1I).

Verifying the Reporter with Known Secretagogues

To determine if the fusion construct could reliably report insulin secretion across a broad range of stimuli, we applied a panel of known secretagogues to cells expressing the reporter, including small molecules, metabolites, and hormones known to affect insulin secretion. Luciferase secretion increased as predicted for each treatment (Figure 2A). The depolarizing agents, potassium chloride and glipizide, triggered insulin secretion even in the absence of glucose, though glucose amplified the amount of secretion in a concentration-dependent manner. In contrast, a glucokinase activator increased secretion primarily in the presence of permissive glucose levels, left-shifting the glucose response curve of the cells, but not affecting their maximal response. The amino acid arginine exhibited a modest glucose-dependent effect on luciferase secretion, as did the incretin hormone glucagon-like peptide-1 (GLP-1). Increasing levels of the secondary messenger cyclic adenosine monophosphate (cAMP) using either IBMX or forskolin led to a profound increase in luciferase secretion at higher glucose levels. Likewise, phorbol 12-myristate 13-acetate (PMA), a compound known to augment insulin secretion through stimulatory effects on protein kinase C, had a large effect on secretion. Luciferase activity remained highly correlated with secreted insulin as measured by ELISA (Figure 2B).

The reporter was further evaluated by performing dose-response studies using two well-studied glucose-dependent insulin secretagogues with distinct mechanisms of action: IBMX and a glucokinase activator. As expected, IBMX increased secretion of luciferase from the cells only in the presence of high glucose (Figure 2C). In contrast, the glucokinase activator did not stimulate additional secretion in high glucose, but did

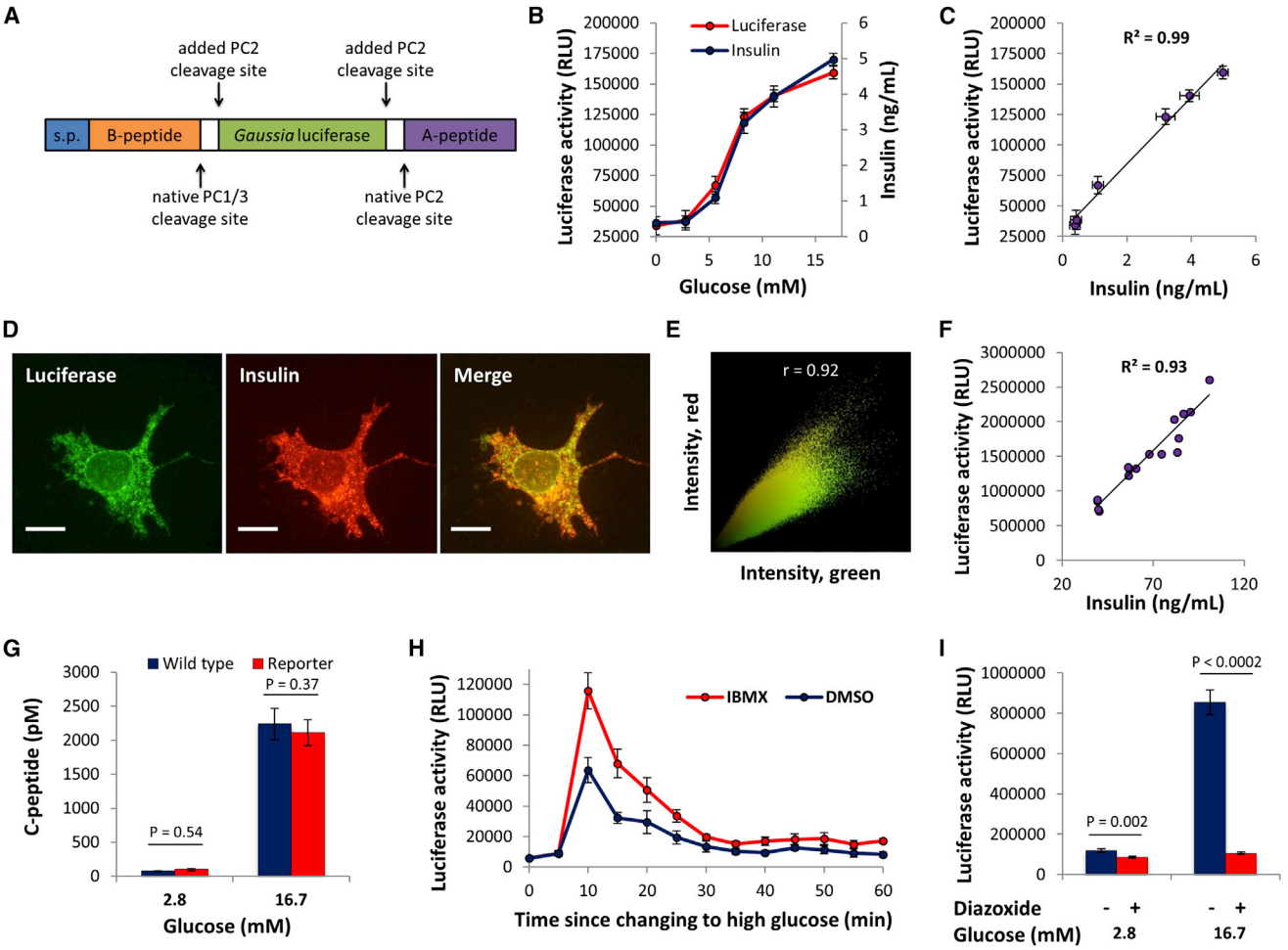

Figure 1. Proinsulin-Luciferase Fusion Protein Faithfully Reports Glucose-Stimulated Insulin Secretion

(A) Diagram of proinsulin-luciferase fusion construct, showing *Gaussia* luciferase without its signal peptide, inserted within the C-peptide portion of mouse proinsulin, with added cleavage sites for prohormone convertase 2 (PC2).

(B) Glucose-stimulated secretion of luciferase (red) and insulin (blue) from INS-1E cells expressing the fusion construct; data represent mean ± SD (n = 8).

(C) Correlation between insulin concentration and luciferase activity of samples shown in (B). See also Figure S1 for correlation in MIN6 cells.

(D) Immunohistochemistry of INS-1E cells expressing the proinsulin-luciferase reporter, stained for luciferase (green, left), insulin (red, middle), or both (merged, right); scale bar, 10 μm.

(E) Quantification of insulin and luciferase colocalization on a pixel-by-pixel basis from INS-1E images in Figure S2.

(F) Correlation between insulin concentration and luciferase activity of dissociated human pancreatic islets expressing the reporter, treated with 2.8 mM, 5.6 mM, 11.1 mM, and 16.7 mM glucose.

(G) Secretion of endogenous C-peptide from wild-type and reporter-containing INS-1E cells; data represent mean ± SD (n = 8).

(H) Time course of glucose-stimulated insulin secretion in INS-1E cells in the presence and absence of known insulin secretagogue IBMX (100 μM). Time zero value corresponds to preincubation in 2.8 mM glucose for 5 min, whereas all subsequent time points correspond to incubation in 16.7 mM glucose; data represent mean ± SD (n = 12).

(I) Effect of diazoxide on luciferase secretion from INS-1E cells, in both basal and glucose-stimulated states; data represent mean ± SD (n = 8).

augment secretion in low glucose when tested at higher concentrations (Figure 2D), consistent with the relative glucose-dependent nature of this class of secretagogue (Rees and Gloyn, 2013).

Tracking Beta-Cell Function under Stress

The toxic environment of the beta cell has been implicated in the pathogenesis of both forms of diabetes, and therapies that counteract this detrimental milieu may reverse the course of disease (Wajchenberg, 2007). To evaluate the luminescent reporter in this setting, beta-cell dysfunction was induced using three established negative mediators: inflammatory cytokines, saturated fatty acids, and ER stress-inducing agents.

Cells expressing the luminescent reporter were treated for 24 hr with a cocktail of cytokines known to be elevated in T2D (Donath et al., 2010), including interleukin-1-beta (IL1β), tumor necrosis factor-alpha (TNFα), and interferon-gamma (IFN-γ), prior to stimulating luciferase secretion with varying glucose concentrations. As expected, luciferase secretion at physiologic and high-glucose levels decreased in proportion to the concentration of cytokines applied to the cells (Figure 2E).

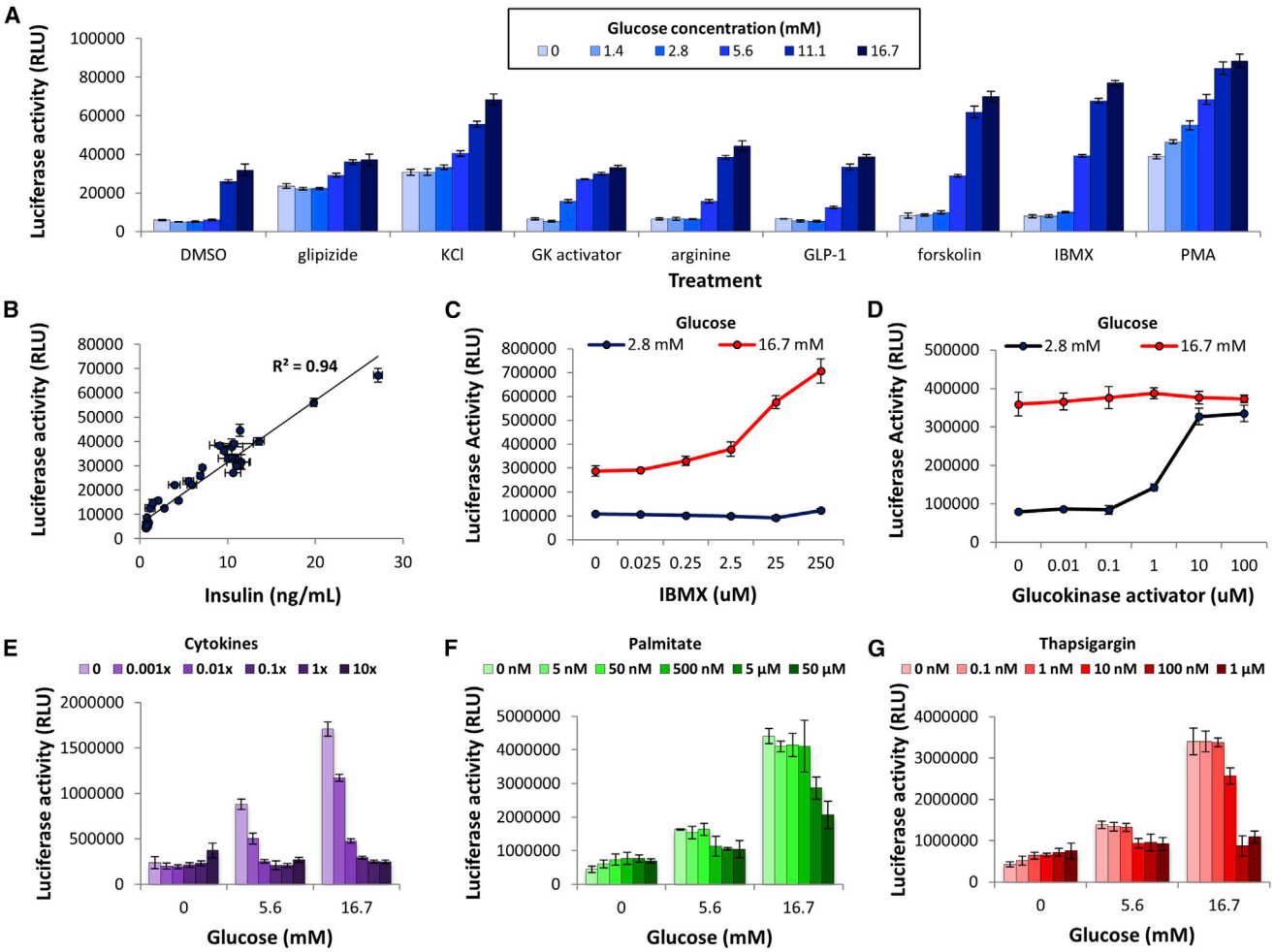

Figure 2. Reporter Responds Appropriately to Known Insulin Secretagogues and Beta-Cell Stressors

(A) Luciferase secretion induced by known insulin secretagogues. MIN6 cells were treated for 1 hr at the indicated glucose concentrations with either DMSO, 2.5 μM glucokinase (GK) activator, 25 μM glipizide, 40 mM potassium chloride (KCl), 20 mM arginine, 1 nM glucagon-like peptide-1 (GLP-1), 20 μM forskolin, 50 μM IBMX, or 20 μM phorbol 12-myristate 13-acetate (PMA); data represent the mean ± SD (n = 4).

(B) Correlation between insulin and luciferase secretion as shown in (A) from MIN6 cells treated with DMSO, glipizide, KCl, GK activator, arginine, and GLP-1; data represent mean ± SD (n = 4).

(C) Dose-response relationship for IBMX in low (2.8 mM) and high (16.7 mM) glucose in INS-1E cells; data represent mean ± SD (n = 4).

(D) Dose-response relationship for a GK activator in low (2.8 mM) and high (16.7 mM) glucose in INS-1E cells; data represent mean ± SD (n = 4).

(E–G) Effect on glucose-stimulated insulin secretion (GSIS) of pretreating INS-1E cells with increasing concentrations of known beta-cell stressors. Data represent mean ± SD (n = 4). (E) 24 hr pretreatment with cytokine cocktail containing interleukin-1-beta (1× = 10 ng/ml), tumor necrosis factor-alpha (1× = 25 ng/ml), interferon-alpha (1× = 50 ng/ml). (F) 24 hr pretreatment with the saturated fatty acid palmitate at various concentrations. (G) 8 hr pretreatment with thapsigargin, a known inducer of ER stress, at various concentrations.

Next, lipotoxicity was induced in the reporter cell line using the saturated fatty acid palmitate. Cells were pretreated with a low dose of palmitate for 24 hr to induce lipotoxicity, as chronic exposure to free fatty acids has been implicated in impaired insulin secretion (Hoppa et al., 2009). Upon stimulation of the cells, luciferase secretion was slightly increased at low-glucose levels and profoundly decreased at higher glucose levels (Figure 2F), consistent with published reports (Elks, 1993).

Finally, the effect of ER stress on luciferase release was tested using thapsigargin, a known inhibitor of the sarcoplasmic and ER calcium channel (SERCA; Lytton et al., 1991). After 8 hr of treatment with this compound, glucose-stimulated luciferase secretion dropped substantially and in a dose-dependent manner (Figure 2G).

Adapting the Reporter to High-Throughput Screening Applications

To enable large-scale chemical and genetic experiments on insulin secretion, the luminescence assay was adapted to 384-well and 1,536-well formats, using a streamlined approach that limited liquid handling steps by performing the assay on cells in suspension. Though secretion from cells in suspension is less than from adherent cells (Figure S3), rodent beta-cell lines respond reproducibly to stimuli in this format, enabling glucose

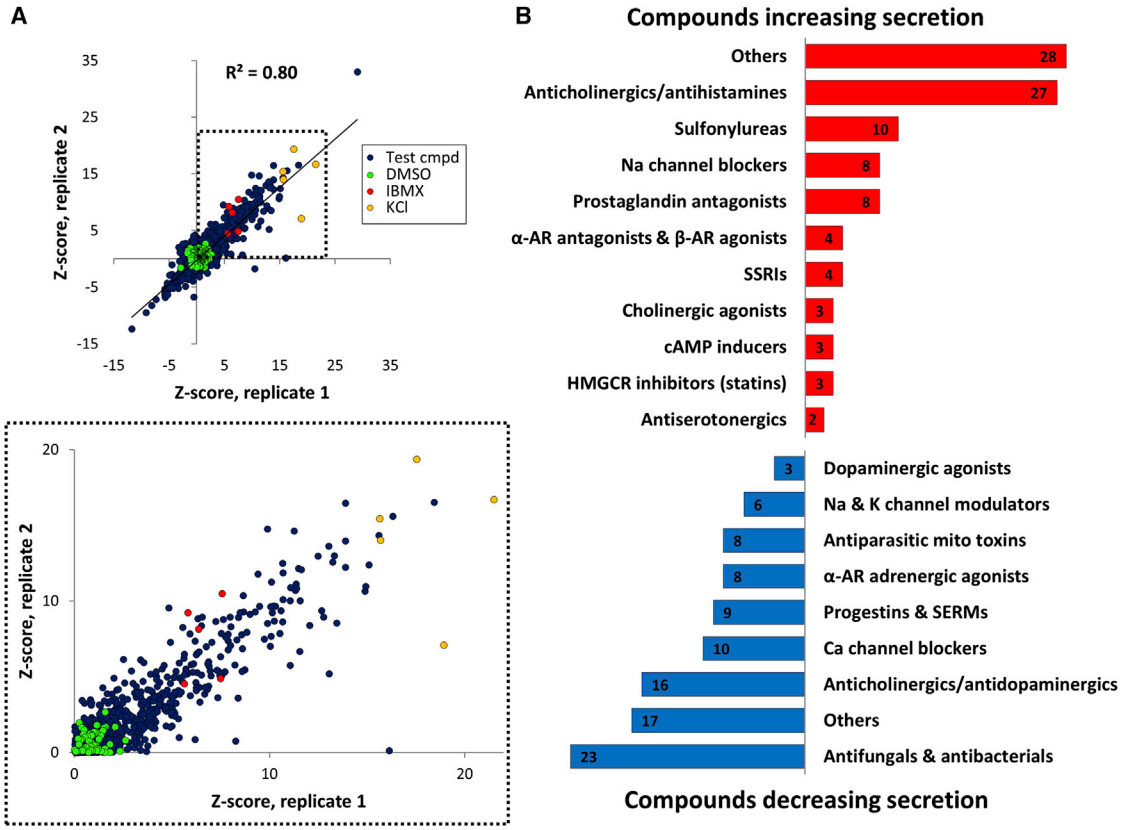

Figure 3. High-Throughput Chemical Screen Performed in Low Glucose Identifies Compounds that Trigger or Inhibit Insulin Secretion

(A) Results of a high-throughput screen of 1,600 known bioactive compounds for effects on luciferase secretion from INS-1E cells expressing the proinsulin-luciferase construct, in the presence of low glucose (2.8 mM), with linear correlation between replicates (r^2 = 0.80); test compounds (blue), DMSO negative controls (green), IBMX (100 μM) positive control (red), KCl (30 μM) positive control (orange). Boxed area shown in greater detail below.

(B) Analysis by class of the 100 top-scoring (red) and 100 bottom-scoring (blue) compounds in the screen, based on reported mechanisms. Number of compounds in each class is shown. α-AR, alpha-adrenergic receptor; β-AR, beta-adrenergic receptor; Na, sodium; K, potassium; Ca, calcium; SSRI, selective serotonin reuptake inhibitor; cAMP, cyclic adenosine monophosphate; HMGCR, 3-hydroxy-3-methyl-glutaryl-CoA reductase; SERM, selective estrogen receptor modulator. See also Figure S3 for comparison of secretion from suspension versus adherent INS-1E cells.

starvation and wash steps to be performed prior to plating cells, which greatly reduces well-to-well variability. In addition, as more cells can be plated per well in suspension, the signal intensity per well is increased significantly.

In this format, secreted luciferase can be measured either by directly adding the substrate coelenterazine to each well in a homogenous manner, or by transferring the supernatant into an empty plate before adding coelenterazine. The transfer step decreases the background signal, yet the assay still performs well without this step, with ample separation of positive and negative controls. For comparison, the Z' factor of the assay (Zhang et al., 1999) is > 0.6 when performed in 384-well format with a transfer step, > 0.5 in 384-well format without a transfer step, and > 0.35 in 1,536-well format without a transfer step, using IBMX as the positive control and a glucose concentration of 11.1 mM.

Performing a Pilot Chemical Screen in Low Glucose

After establishing the suitability of the luminescent reporter for high-throughput applications, we performed a pilot small molecule screen in INS-1E cells using a collection of 1,600 known bioactive compounds and natural products, using IBMX and potassium chloride as positive controls. Each compound was first tested in relatively low glucose (2.8 mM) in duplicate, with high reproducibility (r^2 = 0.80), and clear separation of the positive and negative controls (Figure 3A).

When ranked by mean Z score, approximately 300 compounds increased secretion of luciferase compared to DMSO controls (Table S1, top). Of these, 48 had a greater Z score than IBMX, and one had a greater Z score than potassium chloride. Several classes of compounds were enriched among the top-scoring 100 small molecules, including the following: anticholinergics/first-generation antihistamines, sodium channel blockers, and prostaglandin antagonists (Figure 3B, in red at top). In addition, all ten of the sulfonylureas present in the library scored within the top 55 compounds, with most falling within the top 25 (Table 1). Numerous small molecules used clinically for conditions other than diabetes and without known links to beta-cell function also scored highly.

At the other end of the spectrum, 130 compounds decreased secretion of luciferase compared to DMSO controls (Table S1, bottom). Classes of small molecules enriched in the bottom-scoring

Table 1. Top-Scoring 25 Compounds from the Low and High Glucose Screens

Screen in Low (2.8 mM) Glucose			Screen in High (8.3 mM) Glucose		
	Compound	Class		Compound	Class
1	tyrothricin	antibiotic	1	colforsin	adenylyl cyclase agonist[b]
2	nateglinide	sulfonylurea[a]	2	milrinone	PDE inhibitor[b]
3	cinromide	antiepileptic	3	benoxinate	local anesthetic
4	glyburide	sulfonylurea[a]	4	levosimendan	calcium sensitizer
5	dyphylline	PDE inhibitor[b]	5	artemisinin	antimalarial
6	glipizide	sulfonylurea[a]	6	anagrelide	PDE inhibitor[b]
7	chlorpropamide	sulfonylurea[a]	7	doramectin	GABAergic agonist
8	meglumine	amino sugar	8	deracoxib	COX-2 inhibitor
9	acetohexamide	sulfonylurea[a]	9	clofarabine	guanosine analog
10	orphenadrine	anticholinergic; antihistamine	10	methacholine	muscarinic agonist
11	simvastatin	HMGCR inhibitor (statin)	11	nialamide	nonselective MAOI
12	betamethasone	glucocorticoid	12	irsogladine	PDE inhibitor[b]
13	triprolidine	anticholinergic; antihistamine	13	estropipate	estrogen + GABAergic agonist
14	chlorpheniramine	anticholinergic; antihistamine	14	famciclovir	guanosine analog
15	aceclidine	muscarinic agonist	15	triamterene	diuretic
16	ipratropium	anticholinergic; antihistamine	16	ornidazole	antifungal
17	repaglinide	sulfonylurea[a]	17	acetarsol	arsenoid
18	levosimendan	calcium sensitizer	18	zaleplon	GABAergic agonist
19	nadide	NAD+	19	cilostazol	PDE inhibitor[b]
20	piroctone olamine	antifungal	20	flopropione	serotonin receptor antagonist
21	phenazopyridine	local anesthetic	21	rofecoxib	COX-2 inhibitor
22	tolbutamide	sulfonylurea[a]	22	phenolphthalein	calcium entry (SOCE) inhibitor
23	phentolamine	alpha-adrenergic antagonist	23	proxyphylline	PDE inhibitor[b]
24	methacholine	muscarinic agonist	24	mefexamide	psychostimulant
25	quinine	K channel antagonist[a]	25	griseofulvin	antifungal

Left: compounds found to increase luciferase secretion in the presence of low (2.8 mM) glucose, as ranked by *Z* score. Right: compounds found to increase luciferase secretion in the presence of high (8.3 mM) glucose, as ranked by *Z* score. PDE, phosphodiesterase inhibitor; HMGCR, 3-hydroxy-3-methyl-glutaryl-CoA reductase; NAD+, nicotinamide adenine dinucleotide; K, potassium; GABA, gamma-aminobutyric acid; COX-2, cyclooxygenase-2; MAOI, monoamine oxidase inhibitor; SOCE, store-operated calcium entry.

[a]Denotes compounds that close potassium channels.

[b]Denotes compounds that increase cAMP.

100 compounds included some expected agents: antifungals/antibacterial agents, antidopaminergics/anticholinergics, calcium channel blockers, alpha-adrenergic receptor agonists, and antiparasitic mitochondrial toxins (Figure 3B, in blue at bottom). Progestin analogs and selective estrogen receptor modulators (SERMs) also inhibited luciferase secretion at this relatively low glucose, as did PPAR-alpha agonists (fibrates) and several dopaminergic agonists.

Repeating Pilot Chemical Screen in High Glucose

To determine if our luminescent assay could be useful for identifying insulin secretagogues that augment insulin release in the setting of hyperglycemia, as required for a treatment for diabetes, we repeated the compound screen in INS-1E cells using a supraphysiologic glucose concentration (8.3 mM), three times higher than the initial screen. As expected, the baseline secretion of luciferase in this assay was increased at this higher glucose level. Similar to the low-glucose setting, high reproducibility was observed between replicates (r^2 = 0.90), with clear separation of positive and negative controls (Figure 4A).

In contrast to the original screen in low glucose, the screen in high glucose identified few compounds that increased secretion of luciferase above baseline. When ranked by mean *Z* score, only 30 compounds increased luciferase secretion compared to DMSO controls, and none scored higher than the positive control, IBMX (Table S2, top). The classes of compounds enriched among these top-scoring small molecules were largely distinct from those identified in the low-glucose screen, including the following: cAMP-inducing agents, GABAergic agonists, ion channel modulators, prostaglandin antagonists, and serotonergic antagonists (Figure 4B, in red at top). Two guanosine analogs also scored highly in the screen, as did one cholinergic agonist (Table 1). Sulfonylureas were notably absent from the top-ranked compounds in this high-glucose screen.

In the high-glucose assay, considerably more compounds decreased secretion of luciferase, with 514 small molecules scoring less than the lowest DMSO control (Table S2, bottom). The classes of compounds enriched at this end of the spectrum were largely similar to the low-glucose screen, with the 100

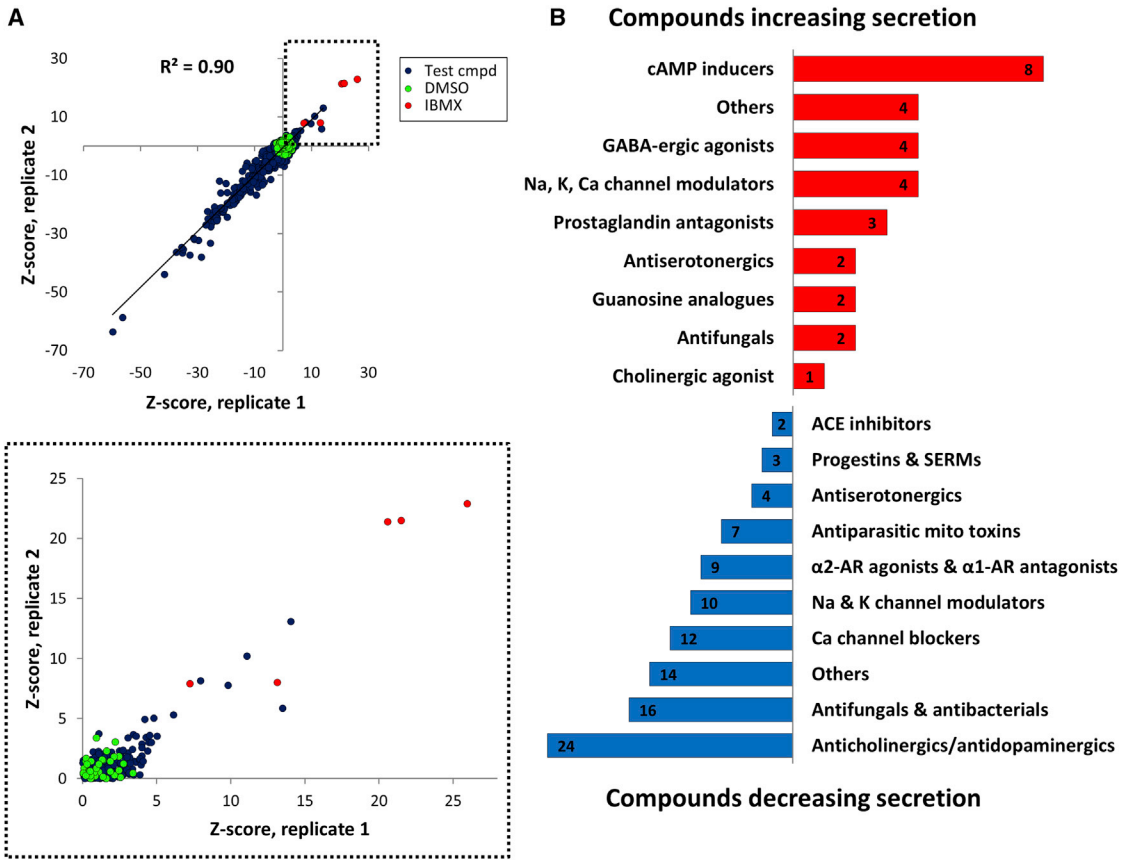

Figure 4. High-Throughput Chemical Screen Performed in High Glucose Identifies Small Molecules that Amplify or Inhibit Insulin Secretion

(A) Results of a high-throughput screen of 1,600 known bioactive compounds for effects on luciferase secretion from INS-1E cells expressing the proinsulin-luciferase construct, in the presence of high glucose (8.3 mM), with linear correlation between replicates (r^2 = 0.90); test compounds (blue), DMSO negative controls (green), IBMX (100 μM) positive control (red). Boxed area shown in greater detail below.

(B) Analysis by class of the 30 top-scoring (red) and 100 bottom-scoring (blue) compounds in the screen, based on reported mechanisms. Number of compounds in each class is shown. Abbreviations as in Figure 3, plus: GABA, gamma-aminobutyric acid; ACE, angiotensin-converting enzyme; α1-AR, alpha-1-adrenergic receptor; α2-AR, alpha-2-adrenergic receptor. See also Figure S4 for comparison of high-throughput screening results in INS-1E and MIN6 cells.

bottom-scoring compounds including the following: anticholinergics/antidopaminergics, antifungals/antibacterial agents, calcium channel blockers, ion channel modulators, antiparasitic mitochondrial toxins, and alpha-2 adrenergic receptor agonists (Figure 4B, in blue at bottom). In addition, several commonly prescribed medications appeared to inhibit glucose-stimulated luciferase secretion, including several angiotensin-converting enzyme (ACE) inhibitors, the PPAR-α agonist fenofibrate, the PPAR-γ agonist pioglitazone, SERMs, progestogens, thyroid hormones, and SSRIs.

To determine if the findings from the high-throughput assays performed in rat INS-1E cells were specific to this cell line or species, we repeated both the low- and high-glucose screens using mouse MIN6 cells expressing the luminescent reporter. A high degree of overlap was observed between the compounds and related drug classes impacting luciferase secretion (Figure S4).

Validating Compounds on Human Islets

To determine if the secretagogues observed in the rodent cell lines were relevant to human biology, we next tested top-scoring small molecules from our high-glucose screen for effects on insulin secretion in human pancreatic islets. To enable a reproducible assessment of compound activity across multiple islets, human islets were gently dissociated and seeded in 96-well format on extracellular matrix (ECM)-coated plates (Walpita et al., 2012), achieving a uniform distribution of beta cells across the plate (Figure S5). The cells remained firmly attached throughout the subsequent wash steps, stimulations, and supernatant harvesting, enabling us to evaluate compounds in parallel with minimal well-to-well variability in beta cell number.

Applying this approach, seven compounds identified in the high-glucose screen as increasing luciferase secretion were tested in dissociated human islets: milrinone, forskolin (equivalent to colforsin), anagrelide, ornidazole, cilostazol, pranoprofen, and benoxinate. Compounds were selected that demonstrated a range of responses in our primary screen, including top hits as well as relatively lower-scoring compounds, to enable an assessment of the sensitivity of the assay in identifying human insulinotropics of various potencies. To evaluate relative glucose dependence, each compound was tested at three glucose levels, 1.67 mM (low), 5.6 mM (normal), and 16.7 mM (high), using dissociated islets from each of four nondiabetic cadaveric donors (Table S5).

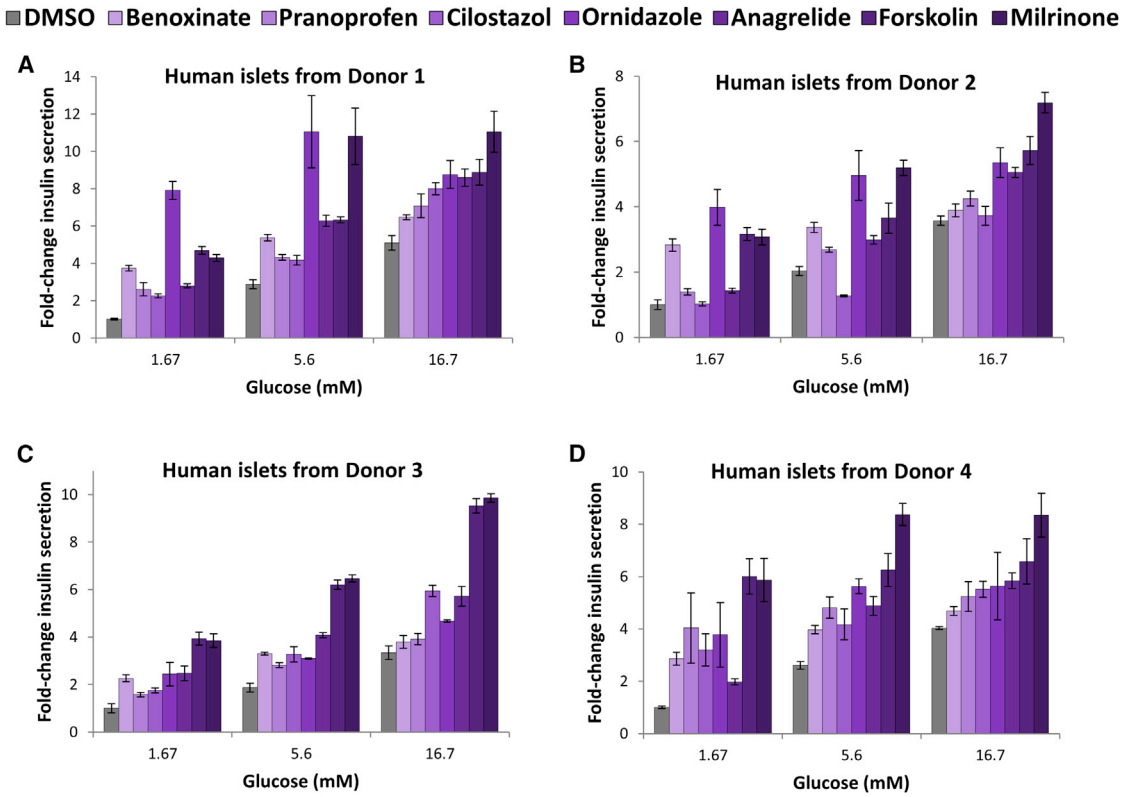

Figure 5. Effects of Compounds from High-Glucose Screen on Insulin Secretion from Human Islets

Seven insulinotropic compounds identified as increasing luciferase secretion from INS-1E cells in the high-glucose screen were tested for effects on insulin secretion from dissociated human islets in the context of three glucose concentrations (1.67 mM, 5.6 mM, 16.7 mM); data represent mean ± SD (n = 4). Compounds are ranked by their effect in 16.7 mM glucose, averaged across donors. (A)–(D) represent four independent, nondiabetic islet donors.

See also Figure S5, demonstrating the homogeneous distribution of dissociated human islets used for compound testing; Figure S6, showing the correlation between compound rank in the INS-1E screen and in human islet experiments; and Table S5, summarizing the human islet donor information.

All seven compounds were found to consistently increase secretion of insulin from human beta cells (Figure 5) in largely the same order of effect as seen in the primary screen in INS-1E cells (Figure S6). While the effect of each compound was potentiated by glucose, none of the compounds were strictly glucose dependent at the relatively high concentrations used in the assay. Milrinone, a phosphodiesterase inhibitor (PDEI) with known links to beta-cell function (Parker et al., 1997), increased insulin secretion more than the other compounds. Forskolin, an activator of adenylyl cyclase, increased secretion in all glucose levels tested. Anagrelide, a PDEI used clinically for essential thrombocytosis, was nearly as effective as forskolin in inducing secretion of insulin at high glucose, but produced less of an effect at low glucose. The remaining compounds, including an antiprotozoal agent (ornidazole), another PDEI (cilostazol), an NSAID (pranoprofen), and a sodium channel antagonist (benoxinate), also augmented insulin secretion in a glucose-potentiated manner.

Investigating the Inhibitory Effect of Fenofibrate on Insulin Secretion

One of the compounds identified in our high-glucose screen that strongly inhibited luciferase secretion, the PPAR-α agonist fenofibrate, is of particular interest because of its widespread clinical use in patients with the metabolic syndrome. To further evaluate the effect of this compound on beta-cell function, we tested it in dose on the INS-1E reporter cell line, across varying glucose concentrations (Figure 6A). The compound dramatically inhibited secretion, in a dose-dependent manner, with more pronounced effects at concentrations approaching its EC_{50} (18 μM in rodent cells). To determine if this effect was relevant to human beta cells, we next tested the compound on dissociated human islets, across varying glucose concentrations, and at concentrations up to its EC_{50} (50 μM in human cells). We observed robust inhibition of GSIS, again in a dose-dependent manner, across multiple independent pancreatic islet donors (Figures 6B and 6C).

Next, to determine if the compound was blocking secretion by acting proximal or distal to the depolarization of the beta cell, we tested its effect on KCl-mediated luciferase secretion from the INS-1E reporter cells. Similar to somatostatin, an established inhibitor of insulin secretion with multiple effects in the beta cell (Hsu et al., 1991), fenofibrate severely inhibited secretion of luciferase under maximal KCl-mediated stimulation (Figure 6D), implying the presence of an effect late in the exocytotic pathway.

Cells treated with fenofibrate during these assays had no obvious morphologic changes, but to more thoroughly evaluate if the compound was toxic to beta cells, we measured the cellular ATP content of fenofibrate-treated INS-1E cells using the CellTiter-Glo reagent (Figure 6E). No significant change in ATP

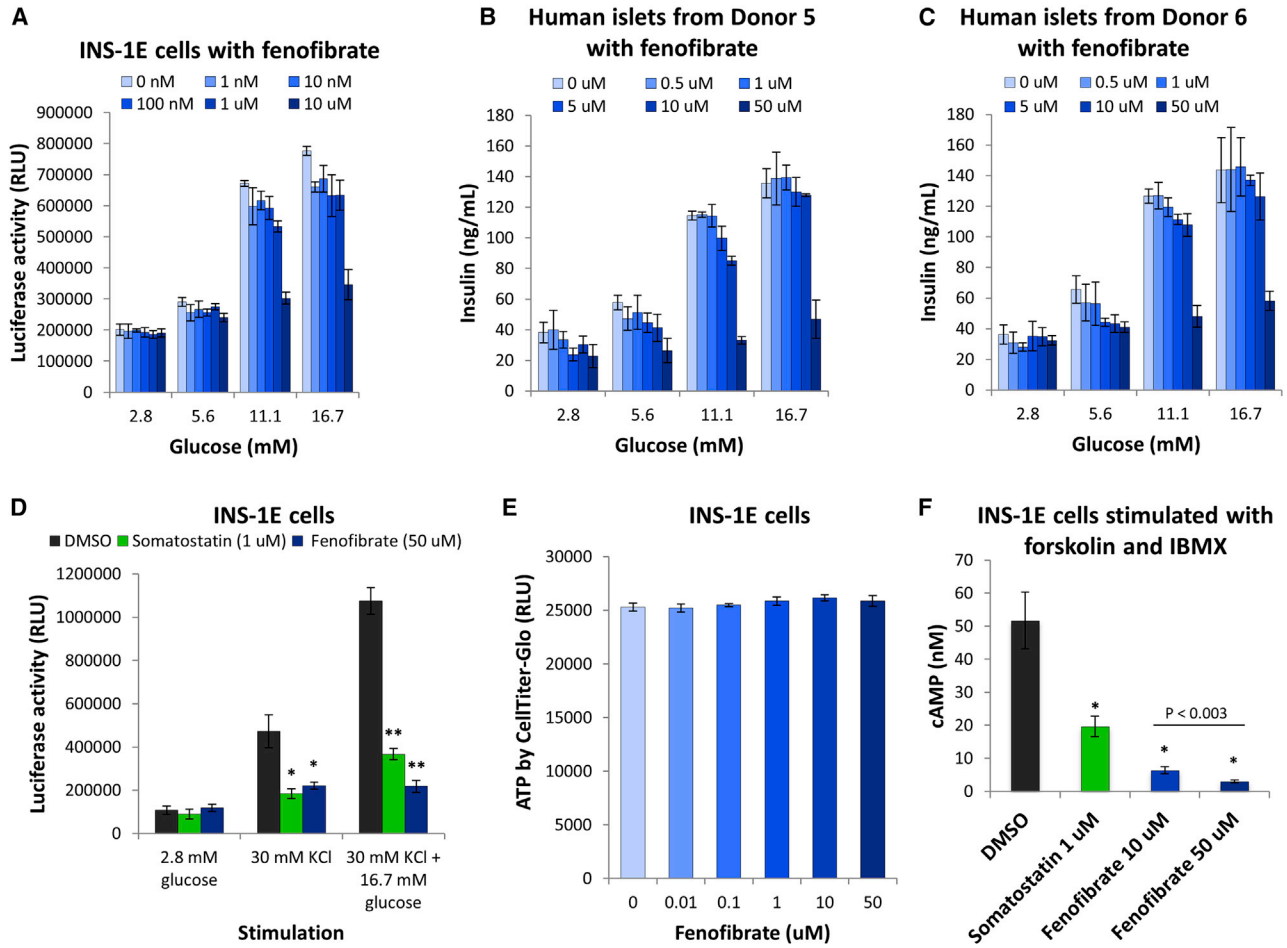

Figure 6. Fenofibrate Decreases Glucose-Stimulated Luciferase Secretion, Insulin Secretion, and cAMP Levels in a Dose-Dependent Manner without Affecting Cellular ATP Levels

(A) Fenofibrate inhibits glucose-stimulated luciferase secretion from INS-1E cells in a dose-dependent manner. The effect of the compound was tested in the context of four glucose concentrations (2.8 mM, 5.6 mM, 11.1 mM, 16.7 mM); data represent mean ± SD (n = 4).

(B and C) Fenofibrate inhibits glucose-stimulated insulin secretion from dissociated human pancreatic islets in a dose-dependent manner; data represent mean ± SD (n = 4). Each plot shows data from an independent, nondiabetic islet donor.

(D) Fenofibrate decreases KCl-stimulated luciferase secretion from INS-1E cells, comparable to somatostatin, a known inhibitor of KCl-stimulated insulin secretion. Data represent mean ± SD (n = 8; two-tailed paired Student's t test in relation to DMSO; * < 10^{-5}, ** < 10^{-8}).

(E) Fenofibrate has no effect on cellular ATP in INS-1E cells, as measured using the CellTiter-Glo assay after 1 hr incubation with varying concentrations of the compound. Data represent mean ± SD (n = 8).

(F) Fenofibrate decreases cAMP levels in a dose-dependent manner, in INS-1E cells stimulated with forskolin (1 μM) and IBMX (500 μM), similar to somatostatin. Data represent mean ± SD (n = 4; two-tailed paired Student's t test in relation to DMSO; * < 0.002). See also Table S5, summarizing the human islet donor information.

content was observed at concentrations up to 50 μM. In contrast, when we measured cAMP levels in cells treated with fenofibrate, we saw a dramatic decrease in this key secondary messenger, greater than the effect of somatostatin (Figure 6F). This effect is likely independent of the compound's agonism of PPAR-α, as other compounds in the same class, including gemfibrozil and 9-oxo-10(E),12(E)-octodecadienoic acid, had no effect on insulin secretion (data not shown).

DISCUSSION

Here we describe the creation, validation, and application of a luminescent insulin secretion reporter that facilitates large-scale assays of beta-cell function. The assay takes advantage of the endogenous proinsulin-processing pathway to target *Gaussia* luciferase to the secretory vesicles within the beta cell. In contrast to the insulin ELISA, the luminescent reporter is fast, inexpensive, and amenable to high-throughput applications in a homogeneous format. In addition to detecting insulin secretagogues, the reporter provides a rapid and straightforward readout of beta-cell dysfunction. As such, it may enable the discovery of compounds or genetic factors protecting against these insults, helping to shed light on the molecular mechanisms by which insulin secretion declines in diabetes.

We demonstrated the utility of the assay by performing pilot screens of 1,600 bioactive compounds and natural products,

in both low and high glucose, and in both rat INS-1E and mouse MIN6 beta-cell lines. While these screens were limited to known bioactives, and thus not designed to discover novel small molecules with activity on beta cells, they may nonetheless help to identify pathways of relevance to human beta-cell function. Indeed, in each glucose level tested, several classes of compounds emerged as augmenting or inhibiting luciferase release, many without prior links to insulin secretion. Numerous compounds increased insulin secretion in the low-glucose screen, whereas very few increased secretion in the more physiologically relevant high-glucose screen, likely a reflection of the distinct mechanisms by which secretion is enhanced in each setting. In low glucose, beta cells are in a polarized resting state, and compounds that shift the membrane potential toward depolarization increase secretion, as suggested by the strong effects of sulfonylureas and sodium channel modulators. In high glucose, however, the beta cells are already depolarized, so compounds that trigger depolarization have minimal additional impact on secretion. In this setting, compounds that augment the amplifying pathway of insulin secretion have more prominent effects, such as cAMP-inducing agents (Almahariq et al., 2014), GABAergic agonists (Bansal et al., 2011), and 5-HT_{1A} serotonin receptor antagonists (Uvnäs-Moberg et al., 1996).

Several intriguing classes of compounds emerged as increasing luciferase secretion, including the following: (in low glucose) anticholinergics/first-generation antihistamines, NSAIDs, and statins; (in high glucose) COX-2 inhibitors, angiotensin receptor blockers (ARBs), and guanosine analogs. Supporting the plausibility of these findings, the M2 and M4 muscarinic receptors (Miguel et al., 2002), as well as certain prostaglandin receptors (Fujita et al., 2007; Kimple et al., 2013), have been reported to negatively regulate insulin secretion. Likewise, statins may increase insulin secretion in low-glucose conditions (Ishikawa et al., 2006), and ARBs improve GSIS in human subjects, reducing the progression to diabetes in subjects with impaired glucose metabolism (van der Zijl et al., 2011). Guanosine analogs may increase the amount of guanosine triphosphate in beta cells, which can directly stimulate insulin secretion (Wollheim and Maechler, 2002).

Our screens also identified classes of compounds that decreased luciferase secretion, including some common medications that have been implicated previously in inhibiting insulin release: SERMs (Le May et al., 2006), progestogens (Shao et al., 2004), SSRIs (Isaac et al., 2013), and the PPAR-gamma agonist pioglitazone (Lamontagne et al., 2009). The PPAR-α agonist fenofibrate, not previously known to affect beta-cell function, was found to robustly block glucose-mediated stimulation of secretion in both rodent and human beta cell models in a dose-dependent manner. Prior reports of fenofibrate's effects on beta cells are conflicting, with one group describing a stimulatory effect on hamster insulinoma cells (Shimomura et al., 2004) and another group demonstrating an inhibitory effect in long-term cultures of mouse islets (Liu et al., 2011). Our results show that the drug acutely decreases insulin secretion at clinically relevant concentrations, possibly through an inhibitory effect on cAMP, and likely independent of its agonism of PPAR-α.

While a number of compounds identified as agonists and antagonists of luciferase secretion in our screen are in clinical use, few have been reported to affect insulin or glucose levels. Possible explanations for this discrepancy include the following: (1) the relatively high concentrations of compounds used in this screen relative to their expected serum levels in patients; (2) the effect of in vivo drug metabolism on compound activity; (3) a limitation of using rodent beta-cell lines and/or isolated cadaveric islets to model complex human biology; (4) as-yet unappreciated side effects of these medications. The luminescent reporter may help to identify effects of medications on beta-cell function that have not been recognized clinically, by creating hypotheses that can be further evaluated using the appropriate clinical and translational research tools (e.g., electronic medical record databases, animal models).

While advantageous for the applications described, the luminescent insulin secretion assay has several limitations. First, it does not detect changes in expression of insulin, but rather, like the ELISA, monitors hormone secretion. Perturbations affecting insulin promoter activity alone, such as those caused by certain beta cell toxins, may not be identified using the reporter. Second, the assay relies on the expression of our fusion construct in an appropriate beta-cell model. We have used well-validated rodent beta-cell lines as surrogates for the human beta cell, yet important differences in beta-cell function exist between species, as well as between transformed cell lines and primary cells. All results must therefore be confirmed using primary human islets and an insulin immunoassay, as we have done. Notably, the reporter appears to function well in human beta cells, and continued efforts to generate functional human beta-cell lines offer the potential to deploy our reporter in an even more relevant biological context. Third, the expression of the luminescent reporter itself may impact aspects of beta-cell function in subtle ways that could influence experimental results. Though we confirmed that C-peptide secretion from reporter-containing INS-1E cells is similar to that from wild-type cells, we cannot rule out an effect of the constitutive expression of the fusion protein on other functional readouts, such as the ER stress response.

In summary, we have shown that a fusion protein in which *Gaussia* luciferase is placed within the C-peptide portion of the insulin prohormone can be used to create a high-throughput luminescent assay of beta-cell function, enabling large-scale experiments that have historically been impractical. Application of this assay to larger, unbiased compound screens may help to characterize the molecular mechanisms governing insulin release, as well as to identify therapeutic leads for preserving and improving beta-cell function in diabetes. Additional reporters based on prohormone-luciferase fusion proteins may also prove useful to monitor the secretion of other peptide hormones relevant to metabolic disease for which immunoassays are currently limiting.

EXPERIMENTAL PROCEDURES

Cell Culture

MIN6 cells were a gift from Dr. Jun-ichi Miyazaki (Osaka University) and were maintained in DMEM with 4.5 g/l glucose, supplemented with 10% heat-inactivated fetal bovine serum and 55 μM beta-mercaptoethanol (Sigma). INS-1E cells were a gift from Dr. Claes Wollheim and Dr. Pierre Maechler (University of Geneva) and were maintained in RPMI with 2 g/l glucose, supplemented with 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 2 mM L-glutamine, 10 mM HEPES, and 55 μM β-mercaptoethanol (Invitrogen). Cells were grown on tissue culture-treated plastic and dissociated with TrypLE (Invitrogen).

Proinsulin-Luciferase Construct

The proinsulin-luciferase fusion construct was created by Gibson Assembly in the pUC19 vector using two gBlocks (Integrated DNA Technologies) encoding the protein and Gibson Assembly Master Mix (New England Biolabs). The proinsulin-luciferase fusion construct was subsequently PCR-amplified with primers to add *attB1* and *attB2* sites, inserted by BP Clonase II into the Gateway Entry vector pDONR223 (Invitrogen), and then shuttled by LR Clonase II into the Gateway Destination vector pLX304 (Yang et al., 2011; Addgene plasmid 25890).

Lentiviral Infection

Lentivirus expressing each prohormone-luciferase fusion protein was produced using a second-generation viral packaging system and used to infect rodent beta-cell lines or human islets, as described in the Supplemental Experimental Procedures.

Immunofluorescence

Cultures were fixed, permeabilized, and stained with antibodies to *Gaussia* luciferase (NanoLight) and insulin (Sigma), as described in the Supplemental Experimental Procedures. Cultures were then incubated in secondary antibodies (Alexa Fluor-conjugated anti-rabbit and anti-guinea pig; Invitrogen), and images were acquired by confocal microscope.

Secretion Assays

For standard secretion assays, MIN6 and INS-1E cells were plated in 96-well format, preincubated for 1 hr in Krebs Ringer Buffer (KRB) with 2.8 mM glucose, and then stimulated for 1 hr in fresh KRB containing varying amounts of glucose and compounds, as described in the Supplemental Experimental Procedures. Insulin or C-peptide concentration was determined using an ELISA (Mercodia), following the manufacturer's protocol. Luciferase activity was determined from the same samples by adding the coelenterazine substrate (NanoLight) to the supernatant to a final concentration of 10 μM and reading on a standard plate reader (BioTek).

Inducing Beta-Cell Dysfunction

INS-1E cells were treated with cytokines, palmitate, or the ER stress-inducing agent thapsigargin to induce cellular dysfunction. For cytokine treatment, we used a mixture that included 10 ng/ml of IL-1β, 50 ng/ml of IFN-γ, and 25 ng/ml of TNF-α (R&D Systems), referred to as the "1X" concentration, dissolved in standard INS-1E growth medium. For palmitate treatment, we dissolved the fatty acid (Sigma) to 50 mM in 90% ethanol at 60°C for several hours, and then diluted this stock in RPMI containing 1% BSA, with all samples receiving the same amount of BSA. For thapsigargin treatment, we dissolved the compound (Sigma) to 1 mM in DMSO and then diluted this stock solution in RPMI containing 1% BSA. Cells were incubated for 24 hr in cytokines and palmitate, or 8 hr in thapsigargin, prior to performing the GSIS assay.

Small Molecule Screens

MIN6 and INS-1E cells were expanded to 80% confluence in their respective growth media, washed once in PBS, and preincubated for 1 hr at 37°C in KRB with 2.8 mM glucose. Cells were then dissociated, spun at 300 × g for 2 min, resuspended in fresh KRB without glucose, and filtered through 40 μm mesh (Becton Dickinson). Next, the cells were counted and diluted in KRB to 1×10^6 cells per ml, and glucose was added as required for each experiment. Cells were then seeded in 384-well format, 3×10^4 cells in 30 μl per well, using a Multidrop Combi device (Thermo Scientific). Compounds from the Pharmakon 1,600 Collection (MicroSource Discovery Systems) were pinned into each well using a Vario robot (CyBio) to a final concentration of 30 μM, and the plates were then incubated for 2 hr at 37°C. For assays involving a transfer step, the plates were then centrifuged at 300 × g for 2 min, and 20 μl of supernatant was transferred to a new 384-well plate. Coelenterazine substrate was then added to a final concentration of 10 μM, and luciferase activity was determined using a standard plate reader (BioTek).

Fenofibrate Investigation

Fenofibrate, gemfibrozil, and 9-oxo-10(E),12(E)-octodecadienoic acid (Cayman Chemical) were resuspended in DMSO, as were forskolin and IBMX (Sigma). Somatostatin-14 (Sigma) was resuspended in sterile water. CellTiter-Glo (Promega) and the cAMP Dynamic-2 HTRF assay (Cisbio) were used according to the manufacturers' instructions. Luciferase secretion and CellTiter-Glo experiments were performed using a 1 hr incubation with compound, whereas the cAMP assay was performed using a 30 min incubation. Secretion experiments were run in KRB buffer; all other experiments were run in standard INS-1E medium.

Statistical Analysis

For the high-throughput screens, a row-based correction factor was applied to all luciferase readings to adjust for logarithmic signal decay, and then a score similar to the statistical *Z* score for each test compound was calculated, as described in the Supplemental Experimental Procedures.

Human Islet Cell Culture

Human islets were obtained through the Integrated Islet Distribution Program (http://iidp.coh.org) and from Prodo Labs (Table S5). Islets were maintained and dissociated in 96-well format as described (Walpita et al., 2012). To test compounds for their effects on insulin secretion, the dissociated islets were washed gently in KRB and preincubated for 1 hr at 37°C in KRB with 2.8 mM glucose. The cells were then washed twice with zero-glucose KRB and placed in 100 μl of fresh KRB with varied glucose concentrations plus compounds. After a 1 hr incubation at 37°C, the supernatant was removed, and insulin concentration was measured by ELISA (Mercodia) according to the manufacturer's instructions.

SUPPLEMENTAL INFORMATION

Supplemental Information includes six figures, five tables, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cmet.2014.12.010.

AUTHOR CONTRIBUTIONS

S.M.B. and A.V. conceived of and designed the constructs; C.K. helped optimize the high-throughput assay; S.M.B. performed the secretion experiments, compound screening, and validation in human islets; D.W. created the human islet culture system and performed the immunohistochemistry; V.D. and P.A.C. aided with the statistical analysis; S.M.B. wrote the manuscript; J.P., P.A.C., B.K.W., and D.A. edited the manuscript.

ACKNOWLEDGMENTS

The work was conducted as part of the Slim Initiative for Genomic Medicine, a project funded by the Carlos Slim Foundation in Mexico. A patent has been filed for the insulin secretion reporter. We thank colleagues at the Massachusetts General Hospital, Broad Institute, and Joslin Diabetes Center for helpful conversations, in particular members of the D.A., Daly, and B.K.W. laboratories, as well as Joseph Avruch, Stuart Schreiber, Nicola Tolliday, Thomas Hasaka, Bakhos Tannous, Gordon Weir, and Susan Bonner-Weir.

Received: April 28, 2014
Revised: October 14, 2014
Accepted: December 13, 2014
Published: January 6, 2015

REFERENCES

Almahariq, M., Mei, F.C., and Cheng, X. (2014). Cyclic AMP sensor EPAC proteins and energy homeostasis. Trends Endocrinol. Metab. *25*, 60–71.

Bansal, P., Wang, S., Liu, S., Xiang, Y.Y., Lu, W.Y., and Wang, Q. (2011). GABA coordinates with insulin in regulating secretory function in pancreatic INS-1 β-cells. PLoS ONE *6*, e26225.

Bielefeld-Sevigny, M. (2009). AlphaLISA immunoassay platform— the "no-wash" high-throughput alternative to ELISA. Assay Drug Dev. Technol. *7*, 90–92.

Boyle, J.P., Thompson, T.J., Gregg, E.W., Barker, L.E., and Williamson, D.F. (2010). Projection of the year 2050 burden of diabetes in the US adult population: dynamic modeling of incidence, mortality, and prediabetes prevalence. Popul. Health Metr. *22*, 29.

Degorce, F. (2006). HTRF(®): pioneering technology for high-throughput screening. Expert Opin. Drug Discov. *1*, 753–764.

Donath, M.Y., Böni-Schnetzler, M., Ellingsgaard, H., Halban, P.A., and Ehses, J.A. (2010). Cytokine production by islets in health and diabetes: cellular origin, regulation and function. Trends Endocrinol. Metab. *21*, 261–267.

Elks, M.L. (1993). Chronic perifusion of rat islets with palmitate suppresses glucose-stimulated insulin release. Endocrinology *133*, 208–214.

Fonseca, V.A. (2009). Defining and characterizing the progression of type 2 diabetes. Diabetes Care *32* (*Suppl 2*), S151–S156.

Fujita, H., Kakei, M., Fujishima, H., Morii, T., Yamada, Y., Qi, Z., and Breyer, M.D. (2007). Effect of selective cyclooxygenase-2 (COX-2) inhibitor treatment on glucose-stimulated insulin secretion in C57BL/6 mice. Biochem. Biophys. Res. Commun. *363*, 37–43.

Hoppa, M.B., Collins, S., Ramracheya, R., Hodson, L., Amisten, S., Zhang, Q., Johnson, P., Ashcroft, F.M., and Rorsman, P. (2009). Chronic palmitate exposure inhibits insulin secretion by dissociation of Ca(2+) channels from secretory granules. Cell Metab. *10*, 455–465.

Hsu, W.H., Xiang, H.D., Rajan, A.S., Kunze, D.L., and Boyd, A.E., 3rd. (1991). Somatostatin inhibits insulin secretion by a G-protein-mediated decrease in Ca2+ entry through voltage-dependent Ca2+ channels in the beta cell. J. Biol. Chem. *266*, 837–843.

Ingelsson, E., Langenberg, C., Hivert, M.F., Prokopenko, I., Lyssenko, V., Dupuis, J., Mägi, R., Sharp, S., Jackson, A.U., Assimes, T.L., et al.; MAGIC Investigators (2010). Detailed physiologic characterization reveals diverse mechanisms for novel genetic loci regulating glucose and insulin metabolism in humans. Diabetes *59*, 1266–1275.

Isaac, R., Boura-Halfon, S., Gurevitch, D., Shainskaya, A., Levkovitz, Y., and Zick, Y. (2013). Selective serotonin reuptake inhibitors (SSRIs) inhibit insulin secretion and action in pancreatic β cells. J. Biol. Chem. *288*, 5682–5693.

Ishikawa, M., Okajima, F., Inoue, N., Motomura, K., Kato, T., Takahashi, A., Oikawa, S., Yamada, N., and Shimano, H. (2006). Distinct effects of pravastatin, atorvastatin, and simvastatin on insulin secretion from a beta-cell line, MIN6 cells. J. Atheroscler. Thromb. *13*, 329–335.

Kimple, M.E., Keller, M.P., Rabaglia, M.R., Pasker, R.L., Neuman, J.C., Truchan, N.A., Brar, H.K., and Attie, A.D. (2013). Prostaglandin E2 receptor, EP3, is induced in diabetic islets and negatively regulates glucose- and hormone-stimulated insulin secretion. Diabetes *62*, 1904–1912.

Lamontagne, J., Pepin, E., Peyot, M.L., Joly, E., Ruderman, N.B., Poitout, V., Madiraju, S.R., Nolan, C.J., and Prentki, M. (2009). Pioglitazone acutely reduces insulin secretion and causes metabolic deceleration of the pancreatic beta-cell at submaximal glucose concentrations. Endocrinology *150*, 3465–3474.

Le May, C., Chu, K., Hu, M., Ortega, C.S., Simpson, E.R., Korach, K.S., Tsai, M.J., and Mauvais-Jarvis, F. (2006). Estrogens protect pancreatic beta-cells from apoptosis and prevent insulin-deficient diabetes mellitus in mice. Proc. Natl. Acad. Sci. USA *103*, 9232–9237.

Lee, J.A., Chu, S., Willard, F.S., Cox, K.L., Sells Galvin, R.J., Peery, R.B., Oliver, S.E., Oler, J., Meredith, T.D., Heidler, S.A., et al. (2011). Open innovation for phenotypic drug discovery: the PD2 assay panel. J. Biomol. Screen. *16*, 588–602.

Liu, M., Hodish, I., Rhodes, C.J., and Arvan, P. (2007). Proinsulin maturation, misfolding, and proteotoxicity. Proc. Natl. Acad. Sci. USA *104*, 15841–15846.

Liu, S.N., Liu, Q., Li, L.Y., Huan, Y., Sun, S.J., and Shen, Z.F. (2011). Long-term fenofibrate treatment impaired glucose-stimulated insulin secretion and up-regulated pancreatic NF-kappa B and iNOS expression in monosodium glutamate-induced obese rats: is that a latent disadvantage? J. Transl. Med. *9*, 176.

Lytton, J., Westlin, M., and Hanley, M.R. (1991). Thapsigargin inhibits the sarcoplasmic or endoplasmic reticulum Ca-ATPase family of calcium pumps. J. Biol. Chem. *266*, 17067–17071.

Merglen, A., Theander, S., Rubi, B., Chaffard, G., Wollheim, C.B., and Maechler, P. (2004). Glucose sensitivity and metabolism-secretion coupling studied during two-year continuous culture in INS-1E insulinoma cells. Endocrinology *145*, 667–678.

Miguel, J.C., Abdel-Wahab, Y.H., Mathias, P.C., and Flatt, P.R. (2002). Muscarinic receptor subtypes mediate stimulatory and paradoxical inhibitory effects on an insulin-secreting beta cell line. Biochim. Biophys. Acta *1569*, 45–50.

Miyazaki, J., Araki, K., Yamato, E., Ikegami, H., Asano, T., Shibasaki, Y., Oka, Y., and Yamamura, K. (1990). Establishment of a pancreatic beta cell line that retains glucose-inducible insulin secretion: special reference to expression of glucose transporter isoforms. Endocrinology *127*, 126–132.

Oyer, P.E., Cho, S., Peterson, J.D., and Steiner, D.F. (1971). Studies on human proinsulin. Isolation and amino acid sequence of the human pancreatic C-peptide. J. Biol. Chem. *246*, 1375–1386.

Parker, J.C., VanVolkenburg, M.A., Nardone, N.A., Hargrove, D.M., and Andrews, K.M. (1997). Modulation of insulin secretion and glycemia by selective inhibition of cyclic AMP phosphodiesterase III. Biochem. Biophys. Res. Commun. *236*, 665–669.

Pouli, A.E., Kennedy, H.J., Schofield, J.G., and Rutter, G.A. (1998). Insulin targeting to the regulated secretory pathway after fusion with green fluorescent protein and firefly luciferase. Biochem. J. *331*, 669–675.

Rees, M.G., and Gloyn, A.L. (2013). Small molecular glucokinase activators: has another new anti-diabetic therapeutic lost favour? Br. J. Pharmacol. *168*, 335–338.

Shao, J., Qiao, L., and Friedman, J.E. (2004). Prolactin, progesterone, and dexamethasone coordinately and adversely regulate glucokinase and cAMP/PDE cascades in MIN6 beta-cells. Am. J. Physiol. Endocrinol. Metab. *286*, E304–E310.

Shimomura, K., Shimizu, H., Ikeda, M., Okada, S., Kakei, M., Matsumoto, S., and Mori, M. (2004). Fenofibrate, troglitazone, and 15-deoxy-Delta12,14-prostaglandin J2 close KATP channels and induce insulin secretion. J. Pharmacol. Exp. Ther. *310*, 1273–1280.

Suzuki, T., Kondo, C., Kanamori, T., and Inouye, S. (2011). Video rate bioluminescence imaging of secretory proteins in living cells: localization, secretory frequency, and quantification. Anal. Biochem. *415*, 182–189.

Tannous, B.A., Kim, D.E., Fernandez, J.L., Weissleder, R., and Breakefield, X.O. (2005). Codon-optimized *Gaussia* luciferase cDNA for mammalian gene expression in culture and in vivo. Mol. Ther. *11*, 435–443.

Uvnäs-Moberg, K., Ahlenius, S., Alster, P., and Hillegaart, V. (1996). Effects of selective serotonin and dopamine agonists on plasma levels of glucose, insulin and glucagon in the rat. Neuroendocrinology *63*, 269–274.

van der Zijl, N.J., Moors, C.C., Goossens, G.H., Hermans, M.M., Blaak, E.E., and Diamant, M. (2011). Valsartan improves beta-cell function and insulin sensitivity in subjects with impaired glucose metabolism: a randomized controlled trial. Diabetes Care *34*, 845–851.

Wajchenberg, B.L. (2007). beta-cell failure in diabetes and preservation by clinical treatment. Endocr. Rev. *28*, 187–218.

Walpita, D., Hasaka, T., Spoonamore, J., Vetere, A., Takane, K.K., Fomina-Yadlin, D., Fiaschi-Taesch, N., Shamji, A., Clemons, P.A., Stewart, A.F., et al. (2012). A human islet cell culture system for high-throughput screening. J. Biomol. Screen. *17*, 509–518.

Wollheim, C.B., and Maechler, P. (2002). Beta-cell mitochondria and insulin secretion: messenger role of nucleotides and metabolites. Diabetes *51* (*Suppl 1*), S37–S42.

Wu, W., Shang, J., Feng, Y., Thompson, C.M., Horwitz, S., Thompson, J.R., MacIntyre, E.D., Thornberry, N.A., Chapman, K., Zhou, Y.P., et al. (2008). Identification of glucose-dependant insulin secretion targets in pancreatic beta cells by combining defined-mechanism compound library screening and siRNA gene silencing. J. Biomol. Screen. *13*, 128–134.

Yang, X., Boehm, J.S., Yang, X., Salehi-Ashtiani, K., Hao, T., Shen, Y., Lubonja, R., Thomas, S.R., Alkan, O., Bhimdi, T., et al. (2011). A public genome-scale lentiviral expression library of human ORFs. Nat. Methods *8*, 659–661.

Zhang, J.H., Chung, T.D., and Oldenburg, K.R. (1999). A simple statistical parameter for use in evaluation and validation of high throughput screening assays. J. Biomol. Screen. *4*, 67–73.