Neuropeptide Y and somatostatin inhibit insulin secretion through different mechanisms

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Schwetz TA, Ustione A, Piston DW. Neuropeptide Y and somatostatin inhibit insulin secretion through different mechanisms. Am J Physiol Endocrinol Metab 304: E211–E221, 2013. First published December 4, 2012; doi:10.1152/ajpendo.00374.2012.—Pancreatic β-cells regulate glucose homeostasis by secreting insulin in response to glucose elevation and G protein-coupled receptor (GPCR) activation. Neuropeptide Y (NPY) and somatostatin (SST) attenuate insulin secretion through Gi activation of Y1 and SSTR1&5 receptors, respectively. The downstream pathways altered by NPY and SST are poorly understood. Thus, we investigated these underlying mechanisms. NPY and SST increase cellular redox potential, suggesting that their inhibitory effect may not be mediated through metabolic inhibition. NPY does not affect intracellular calcium ([Ca2+]i) activity upon glucose stimulation, whereas SST alters this response. Gαs-subunit inhibition by gallein attenuates insulin secretion but does not alter metabolism or [Ca2+]i. mSIRK-induced Gβγ activation does not modulate glucose metabolism but increases [Ca2+]i activity and potentiates insulin release. Cotreatment with gallein and NPY or SST reduces insulin secretion to levels similar to that of gallein alone. mSIRK and NPY cotreatment potentiates insulin secretion similarly to mSIRK alone, whereas mSIRK and SST treatment decreases insulin release. The data support a model where SST attenuates secretion through Gβγ inhibition of Ca2+ activity, while NPY activates a Ca2+-independent pathway mediated by Gαs. GPCR ligands signal through multiple pathways to inhibit insulin secretion, and determining these mechanisms could lead to novel diabetic therapies.

G protein-coupled receptor; Ca2+ signaling; Gβγ subunit

GLUCOSE HOMEOSTASIS is a tightly regulated process coordinated by insulin and glucagon secretion. In β-cells, increasing extracellular glucose concentrations raise cellular metabolism and increase the ATP/ADP ratio, inhibiting the ATP-sensitive inward rectifying potassium (KATP) channel. KATP channel inhibition depolarizes the cell and activates voltage-gated Ca2+ channels (VGCC), triggering insulin secretion. This process, termed glucose-stimulated insulin secretion (GSIS), is relatively well understood. In addition to this glucose-dependent pathway, it is widely accepted that several broadly expressed G protein-coupled receptor (GPCR) ligands can regulate insulin and glucagon release by mediating intracellular signaling pathways. The neurotransmitter neuropeptide Y (NPY) is expressed in sympathetic neurons that innervate the islet (14), and the islet itself, as mRNA of NPY and its receptor, Y1, was detected in isolated mouse islets (22, 23). Steroid induction of NPY expression accompanied by a decrease in insulin release also has been observed in rat β-cells (29) and RINm5F cells (30). However, other studies have suggested that NPY expression is restricted to neonatal β-cells, as expression may be downregulated throughout development (31, 48). NPY inhibits insulin secretion from β-cells by activation of a Gβγ-dependent effects of two Gβγ modulators: an inhibitor of Gβγ-mediated activities, gallein, and mSIRK, a Gβγ activator. By measuring the glucose signaling and secretion responses during treatment with these compounds alone and in combination, we determined whether NPY and SST mediate changes in insulin secretion through a Gβγ-dependent mechanism.

MATERIALS AND METHODS

Islet isolation. All marine procedures were performed to, and approved by, the Vanderbilt University Institutional Animal Care and Use Committee. The islet isolation protocol was adapted from previously described methods (41, 46). Pancreata from 8- to 12-wk-old C57BL/6 adult mice (Harlan) were excised and digested in 0.15–0.22% collagenase P (Roche) per milliliter of Hank’s balanced salt solution (HBSS, Invitrogen) for 8–12 min under gentle agitation. Samples were centrifuged three times, and the supernatant was replaced with fresh HBSS after each spin. Individual islets were pipetted into fresh media [RPMI 1640 (Invitrogen) supplemented with 10%...
fetal bovine serum (FBS, Invitrogen), penicillin-streptomycin (Invitrogen), and 11 mM glucose] and incubated at 37°C and 5% CO₂ for 24–48 h.

Microfluidic device construction. Microfluidic devices were constructed using Sylgard 184 silicone elastomer base and curing agent mix (Dow Corning), which was degassed and cured on a master mold for 3 h at 75°C. A well and two access holes for loading and removing the islets were created. The molds were bonded onto 22 × 40 mm cover glass (Corning) following plasma cleaning (Harrick Scientific).

NAD(P)H imaging. All imaging experiments were conducted in a microfluidic device at 37°C and 5% CO₂. The imaging buffer consisted of (in mM) 125 NaCl, 5.7 KCl, 2.5 CaCl₂, 2H₂O, 1.5 MgCl₂, 10 HEPES, and 0.1% bovine serum albumin (BSA, Sigma Aldrich) at pH 7.4. NAD(P)H autofluorescence was imaged with an LSM710 microscope (Carl Zeiss) using a Plan-Apochromat 20×/0.8 NA objective and a Coherent Chameleon laser tuned to 710 nm, similar to what was previously described (40). The laser power at the sample was below 3.5 mW to prevent damage to the islet (36). NAD(P)H autofluorescence was measured in intact islets as a function of glucose concentration (2–23 mM) with and without NPY (100 nM; Bachem), SST (1 µM; Sigma Aldrich), NE (1 µM; Calbiochem), gallein (10 µM; Calbiochem), or mSIRK (30 µM; Calbiochem). Addition of increasing glucose concentrations with and without the GPCR ligands/Gαᵣ modulators occurred at 8-min intervals to allow NAD(P)H levels to plateau, after which Z-stacks were collected.

Imaging of Ca²⁺ oscillations. [Ca²⁺]i oscillation frequency was measured pre- and posttreatment with NPY, SST, gallein, or mSIRK individually and in combination. Intact islets were labeled with Fluo-4-AM (4 µM; Invitrogen) in imaging buffer with 2 mM glucose for 30–45 min prior to data collection. Oscillations in Fluo-4-AM fluorescence over the whole islet area were detected by excitation at 488 nm on an LSM 510 microscope (Carl Zeiss) with a Plan-Apochromat 20×/0.8 NA lens. Images were collected at one frame every 2 s to measure the fast oscillations in [Ca²⁺]i, generated by changes in ion channel conductances (~25 s) (6). Cells were imaged at 10 mM glucose for ~5–10 min to allow sufficient time for synchronous oscillations to appear; the GPCR ligand and/or Gαᵣ modulator of interest then was added, and oscillations were continuously recorded for another ~10 min. In the experiment performed at 2 mM glucose, the glucose concentration is below the oscillation threshold. Thus, the Fluo-4-AM fluorescence was collected for 3 min, followed by addition of the GPCR ligand and/or Gαᵣ modulator of interest. The fluorescence was then monitored for the subsequent 5 min. Data were normalized to the untreated control frequency, amplitude, or intensity for each islet prior to addition of a GPCR ligand and/or Gαᵣ modulator.

Static incubation insulin secretion assays. Islets were isolated as described above and allowed to recover overnight. Islets were preincubated for 1 h in KRBH buffer consisting of (in mM) 128.8 NaCl, 4.8 KCl, 1.2 KH₂PO₄, 1.2 MgSO₄·7H₂O, 2.5 CaCl₂, 20 HEPES, and 5 NaHCO₃ (pH7.4) with 2.8 mM glucose. Four islets per sample were incubated in 1 ml of KRBH buffer at low (2.8 mM) or high (16.7 mM) glucose with and without NPY, SST, gallein, mSIRK, mSIRK(L9A; 30 µM; Calbiochem), or nifedipine (10 µM, Sigma) individually or in combination for 45 min at 37°C; each condition was measured in triplicate. The samples were briefly spun at 3,000 rpm (Beckman), and 500 µl of each sample was placed in a new tube. Triton X (500 µl of 2%) was added to each islet sample to lyse the islets prior to storage at −20°C. Insulin content (from the Triton X-containing samples) and secretion were analyzed in duplicate with a Mouse Ultralensensitive Insulin ELISA kit (Alpco) and detected on a Spectra Max M5 spectrometer (Molecular Devices).

Analysis and statistics. Data were analyzed with Microsoft Excel, ImageJ, MatLab, or GraphPad Prism analysis software. For all imaging data, the background signal was subtracted and the mean ± SE was determined. Student’s t-tests and ANOVAs were used where applicable, and P < 0.05 was considered statistically significant unless otherwise noted.

RESULTS

An increase in cellular metabolism is associated with NPY and SST inhibition of insulin release. NPY and SST have previously been shown to attenuate insulin release (12, 19, 28, 35). To verify these results and confirm that this inhibition is observed under our conditions, isolated murine islets were incubated at low (2.8 mM) and high (16.7 mM) glucose levels. Treatment with NPY (100 nM) or SST (1 µM) did not alter insulin secretion at low glucose levels compared with untreated control (0.19 ± 0.08 and 0.38 ± 0.08% vs. 0.19 ± 0.04%, respectively, P > 0.05; Fig. 1A). Similar to previously published results (28, 35), insulin secretion decreased upon treatment with NPY at high glucose levels (0.41 ± 0.03%) compared with untreated control (0.68 ± 0.11%, P < 0.05; Fig. 1A). At high glucose concentrations, SST reduced insulin release (0.34 ± 0.08%) compared with untreated control (0.68 ± 0.11%, P < 0.03; Fig. 1A), consistent with prior studies (12, 19, 34, 39).

Fig. 1. Neuropeptide Y (NPY) and somatostatin (SST) attenuate insulin secretion and increase the cellular redox potential from intact islets. A: percent insulin content secreted from intact islets after static incubation at low (LG, 2.8 mM) or high (HG, 16.7 mM) glucose with and without NPY (100 nM, gray squares), SST (1 µM, black triangles), or NE (1 µM, black diamonds) vs. values at 2 mM glucose. Data are means ± SE; n = 5–11. *P < 0.05, significance compared with untreated control at high glucose levels. B: glucose dose-response curves of percent change in NAD(P)H from untreated intact islets (black circles) and islets treated with NPY (100 nM, gray squares), SST (1 µM, black triangles), or NE (1 µM, black diamonds) vs. values at 2 mM glucose. Data are means ± SE; n = 3–11. NPY, SST, and NE treatment at glucose concentrations between 5 and 23 mM is significant. *P < 0.005.
Table 1. Measured [Ca\textsuperscript{2+}]i oscillation parameters, as detected by Fluo 4-AM imaging

<table>
<thead>
<tr>
<th>GPCR Ligand/G\textsubscript{i} Modulator</th>
<th>n</th>
<th>GPCR Ligand/G\textsubscript{i} Modulator</th>
<th>Frequency, mHz</th>
<th>Amplitude, %Δ</th>
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<tbody>
<tr>
<td>Neuropeptide Y</td>
<td>6</td>
<td>+</td>
<td>36.33 ± 1.7</td>
<td>9.45 ± 2.0</td>
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<tr>
<td>Somatostatin</td>
<td>7</td>
<td>−</td>
<td>34.47 ± 1.9</td>
<td>7.05 ± 1.6</td>
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<tr>
<td>Gallicin</td>
<td>5</td>
<td>+</td>
<td>27.16 ± 2.9*</td>
<td>8.74 ± 1.0</td>
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<tr>
<td>SST</td>
<td>5</td>
<td>+</td>
<td>44.78 ± 7.8</td>
<td>4.23 ± 3.9</td>
</tr>
<tr>
<td>mSIRK</td>
<td>5</td>
<td>+</td>
<td>41.17 ± 7.9</td>
<td>1.32 ± 1.2</td>
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<tr>
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<td>+</td>
<td>37.13 ± 1.3</td>
<td>3.18 ± 0.9</td>
</tr>
<tr>
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<td>+</td>
<td>31.13 ± 1.3</td>
<td>2.95 ± 0.9</td>
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<tr>
<td>SST + mSIRK</td>
<td>4</td>
<td>+</td>
<td>25.49 ± 9.4</td>
<td>17.88 ± 6.1</td>
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</table>

Data are means ± SE for frequency and amplitude of [Ca\textsuperscript{2+}]i oscillations measured pre- and posttreatment with the specified G protein-coupled receptor (GPCR) ligand and/or G\textsubscript{i} modulator. Significance was tested using a two-tailed Student’s t-test to compare the frequency or amplitude between untreated and treated islets. Significance P < 0.04.

Glucose metabolism and insulin secretion are tightly coupled processes. As glucose is metabolized, β-cells produce the reduced pyridine nucleotides NADH and NADPH (collectively denoted NAD(P)H) through glycolysis and the TCA cycle; autofluorescence of these byproducts serves as an index of the cellular redox state and, by extension, metabolism. Cellular redox potential changes, via alterations in NAD(P)H autofluorescence, can be quantitatively measured by two-photon excitation microscopy (2, 36).

Here, we investigated whether the NPY and SST-induced attenuation of insulin secretion results from changes in the cellular redox state. NAD(P)H autofluorescence from intact islets was measured as a function of glucose with and without NPY or SST. In untreated control islets, increasing glucose concentrations produced a rise in the NAD(P)H autofluorescence and cellular redox potential (Fig. 1B), as expected (2). Islets treated with NPY or SST showed an additional increase in NAD(P)H levels compared with untreated control over a range of glucose concentrations (P < 0.005; Fig. 1B). To determine whether this effect is specific to NPY and SST, NAD(P)H autofluorescence was measured upon treatment with another inhibitor of insulin secretion, norepinephrine (NE, 1 μM) (37). NE additionally increased NAD(P)H levels as a function of glucose compared with untreated control, to an even greater extent than NPY and SST. These three ligands attenuated insulin release; thus, the results suggest their inhibitory effect was due to alteration of pathways downstream or independent of glucose metabolism.

NPY and SST differentially affect Ca\textsuperscript{2+} activity. Ca\textsuperscript{2+} activity is tightly coupled to insulin secretion (20, 50). At glucose concentrations above 7 mM, murine islets display synchronous, fast oscillations in β-cell [Ca\textsuperscript{2+}], corresponding to the pulsatile release of insulin (5, 6). Here, we measured the β-cell Ca\textsuperscript{2+} response in intact islets upon treatment with NPY or SST by detecting changes in the fluorescence intensity of the Ca\textsuperscript{2+} indicator dye Fluo 4-AM.

Despite studies showing NPY inhibits L-type Ca\textsuperscript{2+} channels in neuronal (42) and cardiac (10) cells, the putative effects of NPY on β-cell Ca\textsuperscript{2+} activity have not been determined. Thus, we treated intact islets with NPY and measured changes in [Ca\textsuperscript{2+}], oscillations at 2 and 10 mM glucose. Upon addition of NPY at 10 mM glucose, neither the [Ca\textsuperscript{2+}], oscillation frequency nor the amplitude of the oscillating or AC component was significantly altered (P > 0.2; Table 1 and Fig. 2, A and B). At 2 mM glucose, the normalized fluorescence intensity was

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also unchanged after addition of NPY (Fig. 6A). Although contrary to previous studies in other cell types, these data suggest that NPY does not attenuate insulin secretion by inhibiting islet Ca\(^{2+}\) activity.

To determine whether SST mediates its inhibition of insulin release by modulating Ca\(^{2+}\) activity, we measured changes in [Ca\(^{2+}\)], oscillations from intact islets with and without SST. SST decreased the [Ca\(^{2+}\)] oscillation frequency (P < 0.04; Table 1 and Fig. 2, C and D) but, similarly to NPY, did not affect the oscillation amplitude (Fig. 2C), suggesting that SST’s inhibitory effect on insulin release is mediated, at least in part, by alteration of [Ca\(^{2+}\)] oscillation frequency. Similarly, addition of SST did not significantly modulate the Ca\(^{2+}\) response at 2 mM glucose (Fig. 6B).

Gallein and mSIRK differentially alter insulin secretion through a metabolism-independent mechanism. GPCRs are coupled to intracellular G proteins, which are activated upon ligand receptor binding and signal through the G\(_{\alpha}\) or G\(_{\beta\gamma}\) subunit (15, 16). To further determine the pathways altered by NPY and SST, two G\(_{\beta\gamma}\) modulators, gallein and mSIRK, were studied. Gallein is a cell-permeable compound that blocks G\(_{\beta\gamma}\)-dependent activities, while mSIRK is an N-myristoylated G\(_{\beta\gamma}\)-binding peptide that binds to and dissociates G\(_{\beta\gamma}\) without activating G\(_{\alpha}\) (17). Insulin secretion at high glucose concentrations was attenuated upon incubation with gallein (10 \(\mu\)M, P < 0.02; Fig. 3A), similar to that observed for NPY and SST; no effect of gallein was observed at low glucose. Conversely, mSIRK (30 \(\mu\)M) dramatically increased insulin release at low and high glucose levels compared with untreated controls (P < 0.005; Fig. 3A). Incubation with mSIRK(L9A) (30 \(\mu\)M), the inactive analog of mSIRK, yielded secretion amounts at low and high glucose that were comparable to those of untreated control (P > 0.2; data not shown).

Cellular redox potential changes upon treatment with gallein or mSIRK were measured to determine whether they mediate metabolism to exert their opposing effects on insulin release. NAD(P)H autofluorescence was measured at increasing glucose concentrations with and without gallein or mSIRK. Neither gallein nor mSIRK elicited a significant effect on NAD(P)H levels compared with untreated control (Fig. 3B). Thus, the data suggest that gallein and mSIRK modulate insulin release through a pathway independent of glucose metabolism.

The islet Ca\(^{2+}\) response is enhanced by mSIRK but not by gallein. [Ca\(^{2+}\)] oscillation frequency at 10 mM glucose and the fluorescence intensity at 2 mM glucose were measured in the presence and absence of gallein or mSIRK. Gallein did not affect the [Ca\(^{2+}\)] oscillation frequency or the normalized fluorescence intensity (Figs. 4, A and B, and 6). Alternatively, mSIRK, which was previously shown to stimulate Ca\(^{2+}\) release from intracellular stores (17), increased the frequency of [Ca\(^{2+}\)] oscillations (P < 0.05; Fig. 4, C and D). The amplitude of the oscillations was not significantly affected upon treatment with either drug (Fig. 4, A and C), although there was a trend toward a reduction with mSIRK. At 2 mM glucose, the Fluo 4-AM fluorescence intensity was increased upon addition of mSIRK, consistent with previously published results (17).

NPY and SST differentially affect Ca\(^{2+}\) activity in the presence of gallein and mSIRK. To determine whether SST inhibits insulin secretion by altering the islet Ca\(^{2+}\) response via the G\(_{\beta\gamma}\) subunit and to verify that NPY does not signal through this pathway, the [Ca\(^{2+}\)] oscillation frequency at 10 mM glucose and the fluorescence intensity at 2 mM glucose were measured with and without combination treatments of SST, NPY, gallein, and mSIRK. Gallein and NPY cotreatment did not affect the [Ca\(^{2+}\)] oscillation frequency (Fig. 5, A and B) or the fluorescence intensity (Fig. 6A). However, treatment with mSIRK and NPY increased the frequency of [Ca\(^{2+}\)] oscillations and the fluorescence intensity at low glucose, similarly to that observed for mSIRK alone (Figs. 5, C and D, and 6A). The amplitude of the oscillating component was decreased upon mSIRK and NPY treatment, not unlike the effect observed for mSIRK alone. Neither cotreatment with gallein and SST nor with mSIRK and SST modulated the [Ca\(^{2+}\)] oscillation frequency (or amplitude; data not shown). Combination treatment with gallein and SST did not alter the fluorescence intensity at low glucose, but cotreatment with mSIRK and SST resulted in elevated fluorescence intensities compared with untreated control. These data suggest that SST reverses the G\(_{\beta\gamma}\)-dependent stimulatory effect of mSIRK on Ca\(^{2+}\) activity at high glucose (Fig. 5, E–H). NPY inhibits insulin secretion through a G\(_{\beta\gamma}\)-independent pathway. As discussed earlier, NPY attenuates insulin secretion downstream of cAMP (28, 35, 43). To determine whether
NPY attenuates insulin secretion through a G\textsubscript{\textbeta\textgamma}-dependent mechanism, isolated islets were incubated at low and high glucose levels with and without NPY and gallein or mSIRK. NPY and gallein combination treatment reduced insulin secretion at stimulatory glucose levels compared with untreated control (0.37 ± 0.06% vs. 0.68 ± 0.11%, respectively); however, NPY and gallein cotreatment did not alter secretion compared with incubation with gallein alone (Fig. 7A). To further examine whether NPY signals through G\textsubscript{\textbeta\textgamma}, islets were incubated with NPY and mSIRK. No effect of NPY was detected upon activation of G\textsubscript{\textbeta\textgamma} by mSIRK at either glucose concentration (P > 0.4; Fig. 7B). Together, the data suggest that NPY mediates its inhibitory effect on insulin release through a G\textsubscript{\textbeta\textgamma}-independent mechanism.

**Insulin secretion inhibition by SST is mediated through G\textsubscript{\textbeta\textgamma}**. SST inhibits insulin secretion by SSTR stimulation of G proteins. To determine whether this inhibition is mediated through G\textsubscript{\textbeta\textgamma}, we measured insulin secretion from intact islets following incubation with gallein and SST. Insulin release at stimulatory glucose levels was decreased upon gallein and SST cotreatment compared with untreated control (P < 0.03; Fig. 7A). However, gallein and SST treatment showed no significant difference at high glucose concentrations compared with SST only (P > 0.6). Additional inhibition of insulin secretion upon combination treatment was not detected; this, in conjunction with our [Ca\textsuperscript{2+}]\textsubscript{i} data at 10 mM glucose, suggests that SST's effect is mediated primarily through G\textsubscript{\textbeta\textgamma}. To test this further, insulin release at high glucose was measured following cotreatment with mSIRK and SST, and a decrease in secretion was detected compared with mSIRK only (P < 0.03; Fig. 7B). SSTRs have previously been shown to associate with VGCCs (33). Thus, to determine whether SST inhibits insulin secretion by altering VGCC activity, insulin release was measured following treatment with nifedipine, an L-type Ca\textsuperscript{2+} channel blocker, alone or in combination with SST. Secretion decreased under both conditions compared with untreated control; however, no additional decrease was noted upon treatment with nifedipine and SST compared with nifedipine alone (Fig. 7C).

**DISCUSSION**

Although GPCR ligands are known to modulate insulin secretion, little is known about the mechanistic details by which this occurs. Thus, we examined the effect of NPY and SST on cellular metabolism and Ca\textsuperscript{2+} activity in the β-cells of intact islets and determined whether the G\textsubscript{\textbeta\textgamma} complex is the primary G protein subunit that mediates the ligand response.

**Insulin secretion is inhibited through a metabolism-independent mechanism by NPY and SST**. NPY and SST inhibit insulin secretion through a pertussis toxin-sensitive process, suggesting that they transduce their actions via G\textsubscript{\textalpha\textio} proteins (28, 34, 35). In this paper, we confirm that NPY and SST attenuate insulin release from isolated murine islets (Fig. 1A). Similar to previously published data (47), we also find that insulin secretion is not altered by SST treatment at substimulatory glucose concentrations, suggesting that SST-dependent inhibition requires glucose metabolism; this was also confirmed when insulin secretion was induced by mSIRK treatment at low glucose (data not shown). Comparable results were observed with NPY. NPY is an orexigenic peptide that regulates energy homeostasis and stimulates weight gain (45). In the central nervous system, SST inhibits the release of growth hormone, which modulates protein, lipid, and carbohydrate metabolism (9, 27). However, little is known about NPY’s and SST’s effects on β-cell metabolism. We determined that both potentiate β-cell redox potential, and thus the metabolism of glucose (Fig. 1B). A previous study found that SST attenuates glucose metabolism by inhibiting the O\textsubscript{2} consumption rate in MIN6 cells and isolated mouse islets (13). However, O\textsubscript{2} consumption measurements from islets are difficult to interpret. Here, changes in NAD(P)H were measured to determine the cellular metabolism by inhibiting the O\textsubscript{2} consumption rate in MIN6 cells; however, no additional decrease was noted upon treatment with nifedipine and SST compared with nifedipine alone (Fig. 7C).
redox potential, which correlates to metabolism. Thus, the data are not necessarily contradictory, as SST may inhibit O$_2$ consumption and still increase glucose metabolism.

The central dogma of insulin secretion states that an increase in glucose metabolism will shift the ATP/ADP ratio to favor the production of ATP, inhibiting the K$_{ATP}$ channel; this depolarizes the cell and allows for VGCC activation and insulin secretion. Since the NAD(P)H data in the presence of NPY and SST are consistent with a potentiation (not attenuation) of secretion, NPY and SST may alter pathways downstream of glucose metabolism. Similar results were observed for another inhibitor of insulin secretion, NE (Fig. 1B). Secretion is an ATP-dependent process, as granule priming requires ATP hydrolysis (51, 52), so NE, NPY, and SST inhibition of secretion likely increases the amount of available ATP, elevating NAD(P)H levels and leading to the associated rise in metabolic activity detected here. This may not be a general characteristic of all insulin inhibitors, but it is a reasonable explanation for these three, although this postulate remains to be tested. Alternatively, alteration of pathways between NAD(P)H generation and conversion by NE, NPY, and SST cannot be excluded as a possible mechanism. In mouse islets, NPY was shown to promote β-cell replication (11). Consequently, the increase in metabolism we observe with NE, NPY, and SST may be, at least in part, a secondary effect of the increased ATP, resulting in stimulation of β-cell replication.

**NPY utilizes a $G_{i}$-independent pathway to attenuate insulin release.** In β-cells, insulin secretion requires increases in [Ca$^{2+}$], (20, 50), where synchronous, coupled oscillations in [Ca$^{2+}$], elicit pulsatile insulin release (1, 4, 5). Thus, we measured [Ca$^{2+}$], with and without NPY or SST to determine whether these ligands mediate insulin secretion through [Ca$^{2+}$], alteration. Prior studies showed that NPY inhibits L-type Ca$^{2+}$ channels in neuronal (42) and cardiac (10) cells. However, we found no change in the Ca$^{2+}$ response with NPY treatment in β-cells (Figs. 2, A and B, and 6A), suggesting that cell type differences are important factors in NPY signaling. The data support a model where NPY attenuates insulin release through a Ca$^{2+}$-independent pathway. This is further corroborated by the lack of effect noted on [Ca$^{2+}$], oscillations and Fluo 4-AM fluorescence intensity (at low glucose) from islets treated with mSIRK and NPY compared with mSIRK alone (Figs. 5, C and D, and 6A).

To determine whether NPY signaling occurs through the $G_{i}$ complex, we utilized two $G_{i}$ modulators, gallein and mSIRK. Gallein inhibits $G_{i}$ activity and, as shown in Fig. 3A, attenuates insulin release at stimulatory glucose levels. Although the specificity of gallein to different $G_{i}$ subunits remains unknown, it has been shown that gallein binds to and inhibits effector binding to $G_{i}$. Gallein is also a competitor of many $G_{i}$ protein-protein interactions (25). mSIRK, a $G_{i}$-activating peptide, stimulates insulin release (Fig. 3A). These results are contrary to a prior study that found that mSIRK decreases exocytosis in INS832/13 cells (53). However, our data are consistent with mSIRK stimulation of Ca$^{2+}$ release from intracellular stores (17), which would cause an increase in secretion. Cell type differences between primary cultured islets, as we used, and tissue culture models likely contribute to

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**Fig. 6. NPY, SST, and gallein do not alter [Ca$^{2+}$] at low glucose, but mSIRK stimulates [Ca$^{2+}$] release.** Changes in Fluo 4-AM signal recorded at 2 mM glucose with and without NPY (100 nM), SST (1 μM), gallein (10 μM), mSIRK (30 μM), or a combination. A: Fluo 4-AM fluorescence intensity normalized to untreated control (white) before and after treatment with NPY (gray), gallein (white checked), NPY and gallein (gray checked), mSIRK (white striped), or NPY and mSIRK (gray striped). B: normalized Fluo 4-AM fluorescence intensity pre- (white) and posttreatment with SST (black), gallein (black checked), SST and gallein (black striped), mSIRK (white striped), or SST and mSIRK (black striped). Data are means ± SE; n = 5. *P < 0.05; #P < 0.005.
the variability between the results observed here and previously (53). Furthermore, treatment with the inactive analog of mSIRK, mSIRK(L9A), did not affect secretion at low or high glucose, in support of our assumption that peptide activation of G\(\beta\gamma\) is responsible for the increase in insulin release observed with mSIRK incubation. Neither gallein nor mSIRK significantly modulate the NAD(P)H response, so these compounds are unlikely to affect secretion through alteration of metabolism (Fig. 3B).

Next, we determined whether gallein and mSIRK modulate the Ca\(^{2+}\) response. Gallein does not alter [Ca\(^{2+}\)]\(_i\) oscillations or fluorescence intensity at low glucose; however, mSIRK significantly increases [Ca\(^{2+}\)]\(_i\) oscillation frequency (Fig. 4) and fluorescence intensity (Fig. 6), consistent with a previous observation that found mSIRK releases Ca\(^{2+}\) from intracellular stores (17). This release of intracellular Ca\(^{2+}\) likely drives the glucose-independent potentiation of insulin secretion observed in Figs. 3A and 7B. The data suggest that, while mSIRK potentiates insulin secretion by upregulating [Ca\(^{2+}\)]\(_i\), gallein likely inhibits insulin release by altering pathways further downstream, possibly at the level of exocytosis.

We measured insulin release upon incubation at low and high glucose concentrations with NPY and gallein. Cotreatment reduces insulin release at stimulatory glucose levels compared with untreated control, similar to gallein treatment alone (Fig. 7A). If NPY signals through a G\(\beta\gamma\)-dependent pathway, we would predict an attenuation of secretion upon cotreatment with NPY and mSIRK. However, no change was observed with NPY and mSIRK combination treatment compared with mSIRK alone (Fig. 7B), similar to the observed results for Ca\(^{2+}\) activity (Fig. 5, D–F). Since mSIRK stimulates the G\(\beta\gamma\) subunit without activating G\(\alpha\), the data support a model where NPY signaling is mediated through the G\(\alpha\) subunit (Fig. 8). Previously, it was shown that, upon receptor activation, both the G\(\alpha\) and G\(\beta\gamma\) subunits function to mediate the downstream signaling response via activation of various effectors (15, 16). Furthermore, NPY was shown to modulate effectors downstream of cAMP generation signaling through a G\(_i\)-coupled receptor (28, 35, 43). Together, the data suggest that NPY likely inhibits insulin secretion through a G\(\beta\gamma\) and metabolism-independent pathway downstream of cAMP that does not alter Ca\(^{2+}\) (Fig. 8).

**Insulin release is inhibited by SST through a G\(\beta\gamma\)-dependent pathway that alters the Ca\(^{2+}\) response.** In this study, we measured \(\beta\)-cell Ca\(^{2+}\) activity prior to and following application of SST. The islet Ca\(^{2+}\) response to increased glucose, which is closely coupled to insulin secretion, was significantly reduced when treated with SST (Fig. 2, C and D). Using gallein and mSIRK, we determined that SST likely exerts this effect on Ca\(^{2+}\) oscillation frequency at elevated glucose levels through a G\(\beta\gamma\)-dependent mechanism (Figs. 5, E–H, and 7) acting on downstream effectors (26). Although a prior study in dispersed

![Fig. 7. NPY likely acts through a G\(\beta\gamma\)-independent pathway, but SST modulates a mechanism dependent on G\(\beta\gamma\) to attenuate insulin release. Percent insulin content secreted from intact islets after static incubation at high glucose (HG, 16.7 mM) with and without treatment. Untreated control samples are shown in white throughout. A: percent insulin content secreted in the presence and absence of G\(\beta\gamma\) inhibitor gallein (10 μM, white checkered), NPY (gray), or SST (black) alone, or combination treatment with gallein and NPY (100 nM, gray checkered) or SST (1 μM, black checkered) at high glucose. B: percent insulin content secreted at high glucose concentrations with and without G\(\beta\gamma\)-activating peptide mSIRK (30 μM, white striped), NPY (gray), or SST (black) alone, or combination treatment with mSIRK and NPY (100 nM, gray striped) or SST (1 μM, black striped). C: percent insulin content secreted at high glucose with and without SST (1 μM, black), L-type Ca\(^{2+}\) channel blocker nifedipine (1 μM, white bricked), or combination treatment with nifedipine and SST (black bricked). Data are means ± SE; n = 4–10. *P < 0.04, #P < 0.005: significance vs. untreated control.](https://ajpendo.physiology.org/doi/fig/10.1152/ajpendo.00374.2012)
Fig. 8. NPY signals through the $G_\alpha$ subunit, while SST activates the $G_{\beta\gamma}$ complex. Model illustrating the known (A, solid lines) and proposed (B, dashed lines) mechanisms of action for NPY and SST modulation of insulin release. $Y_1$, NPY receptor; SSTRs, SST receptor; VGCC, voltage-gated calcium channel; PLC, phospholipase C; AC, adenylate cyclase; ER, endoplasmic reticulum. Our data suggest that NPY signals through a $G_\alpha$- and metabolism-dependent pathway that does not modulate the Ca$_{2+}$ response; SST inhibits insulin release through a $G_{\beta\gamma}$- and Ca$_{2+}$-dependent pathway that increases metabolism.

β-cells found no effect of SST on Ca$_{2+}$ currents (39), our data are consistent with other published results in HIT and MIN6 cells, which showed that SST decreases the Ca$_{2+}$ influx through VGCCs (21, 44). These inconsistencies may be accounted for by the differences in tissue culture models and the use of dispersed β-cells, which removes them from the islet microenvironment that is required for normal β-cell function (3). In our work, β-cells in intact islets were utilized to minimize these possible complications. Application of mSIRK and SST returned Ca$_{2+}$ oscillation frequency to pretreatment levels (Fig. 5, G and H); however, the Fluo 4-AM fluorescence intensity at low glucose and secretion levels upon combination treatment with these drugs remained elevated compared with untreated control. This could be due to mSIRK stimulation of Ca$_{2+}$-independent pathways that are not altered by SST or by partial SST attenuation of Ca$_{2+}$ release from intracellular stores. Figure 6B also suggests that, consistent with the secretion data, the inhibition of insulin secretion by SST may be glucose dependent.

SSTR isoforms expressed in the β-cell associated with VGCCs and phospholipase C (PLC) (33). Thus, SST may locally inhibit VGCCs and/or PLC to decrease Ca$_{2+}$ influx and/or release of Ca$_{2+}$ from intracellular stores, respectively. As previously mentioned, our mSIRK secretion data suggest that SST may partially attenuate Ca$_{2+}$ release from intracellular stores (Fig. 5, G and H, and 7B). Moreover, the reduced frequency of [Ca$_{2+}$]$_i$ oscillations is consistent with VGCC modulation (4, 7), which would attenuate β-cell secretion. Treatment with the L-type Ca$_{2+}$ blocker nifedipine in combination with SST reduces insulin secretion compared with untreated control but not compared with either compound alone (Fig. 7C). If SST’s effects are mediated through a mechanism independent of these channels, secretion would be further reduced. Thus, SST may inhibit insulin release at stimulatory glucose through alteration of VGCC activity, specifically L-type Ca$_{2+}$ channels. However, SST and nifedipine alone may maximally inhibit secretion and thus render no additional effect upon combination treatment; this possibility cannot be eliminated. Alternatively, secretory granules located near L-type Ca$_{2+}$ channels may be deprimed and calcineurin activated upon SSTR activation, as has been proposed in α-cells (18). SSTRs are also coupled to inward-rectifying K$^+$ channels, including K$_{ATP}$ channels (33). It is possible, therefore, that SSTR activation at high glucose stimulates the $G_{\beta\gamma}$ complex and locally activates K$_{ATP}$ channels, hyperpolarizing the membrane and decreasing Ca$_{2+}$ influx through VGCCs. SST action on VGCCs, K$_{ATP}$ channels, or a combination of these two effects would explain our data. This effect, however, appears to be limited to stimulatory glucose concentrations, when insulin secretion is elevated.

Summary. Our data show that, although NPY and SST both inhibit insulin release, they do so through different mechanisms. NPY binding to its receptor likely acts through the $G_\alpha_\text{subunit}$ to inhibit a Ca$_{2+}$-independent pathway, further downstream of glucose metabolism. Attenuation of the Ca$_{2+}$ response at high glucose upon treatment with SST is mediated through $G_{\beta\gamma}$ complex signaling; our data indicate that this occurs downstream of glucose metabolism and may be through direct alteration of electrophysiological activity and/or intracellular signaling. Decreased NPY and/or SST secretion may be a compensatory method by which the body attempts to overcome the insulin-resistant state of type 2 diabetes. Thus, insight into the mechanism(s) by which these two ligands exert their effects will allow for a more comprehensive understanding of islet function and the pathology of type 2 diabetes. Considering the possible influence of GPCRs on insulin secretion and the abundance of available therapies targeting this receptor class, possible relief of these insulin-inhibiting pathways could lead to novel therapeutic targets for type 2 diabetes.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: T.A.S. and D.W.P. conception and design of research; T.A.S. and A.U. performed experiments; T.A.S. and A.U. analyzed data; T.A.S. and D.W.P. interpreted results of experiments; T.A.S. and A.U. prepared figures; T.A.S. drafted manuscript; T.A.S., A.U., and D.W.P. edited and revised manuscript; T.A.S., A.U., and D.W.P. approved final version of manuscript.

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