Sphingosine-1-phosphate (S1P) regulates glucose-stimulated insulin secretion in pancreatic beta cells

Jamie Cantrell Stanford1, Andrew J. Morris2, Manjula Sunkara2, Gabriel J. Popa1, Kara L. Larson1 and Sabire Özcan1

From the Department of Molecular and Cellular Biochemistry1 and Cardiovascular Research Center2 University of Kentucky, Lexington, KY 40536

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Address correspondence to: Sabire Özcan, 741 S. Limestone St., BBSRB, B155, Lexington, KY 40536
Phone: 859-257-4821; Fax: 859-257-2283; Email: sozcan@uky.edu

Background: Sphingolipids play an important role in glucose homeostasis.

Results: High glucose induces SphK activity, leading to increases in S1P levels and stimulation of insulin secretion.

Conclusion: SphK activity and S1P levels are critical for glucose-stimulated insulin secretion.

Significance: The presented data uncover a new role for SphK and S1P in regulation of insulin secretion by glucose.

Recent studies suggest that sphingolipid metabolism is altered during type 2 diabetes. Increased levels of the sphingolipid ceramide are associated with insulin resistance. However, a role for sphingolipids in pancreatic beta-cell function, or insulin production, and release remains to be established. Our studies in MIN6 cells and mouse pancreatic islets demonstrate that glucose stimulates an intracellular rise in the sphingolipid, sphingosine-1-phosphate (S1P), while the levels of ceramide and sphingomyelin remain unchanged. The increase in S1P levels by glucose is due activation of sphingosine kinase 2 (SphK2). Interestingly, rises in S1P correlate with increased glucose-stimulated insulin secretion (GSIS). Decreasing S1P levels by treatment of MIN6 cells or primary islets with the sphingosine kinase inhibitor SKI reduces GSIS. Moreover, knockdown of SphK2 alone results in decreased GSIS, whereas knockdown of the S1P phosphatase, Sgpp1, leads to a rise in GSIS. Treatment of mice with the sphingosine kinase inhibitor SKI impairs glucose disposal due to decreased plasma insulin levels. Altogether, our data suggest that glucose activates SphK2 in pancreatic beta cells leading to a rise in S1P levels, which is important for GSIS.

The onset and progression of type II diabetes has been linked to alterations in sphingolipid metabolism (1-4). Studies show that ceramide levels are significantly increased in the blood, muscle, liver, and adipose tissue of diabetic rodents and humans (1,5-7). Increased levels of ceramide correlate with increased insulin resistance, suggesting that ceramide plays an important role in promoting insulin resistance (1,2,4,7). Consistent with this idea, inhibition of ceramide synthesis enhances insulin sensitivity (8-10). Recent data indicate that ceramide may also inhibit insulin gene transcription in pancreatic beta cells under diabetic conditions (11,12). In addition, ceramide, which is known induce apoptosis in many cell types, has been shown to mimic cytokine-induced beta-cell death (13,14).

The sphingolipid, sphingosine-1-phosphate (S1P), that opposes the actions of ceramide, also has an effect on beta-cell function. S1P protects beta cells from cytokine-induced cell death (14). S1P may also have a role in insulin secretion. Exogenously added S1P promotes insulin secretion in HIT-T15 cells and mouse pancreatic islets (15). S1P acts as a signaling molecule by binding both extracellular and intracellular targets (16,17). Extracellular targets include the five known S1P receptors, S1P 1,5, four of which (S1P 1-4) are expressed in the rat pancreatic INS1 cell line and mouse pancreatic islets (18). S1P receptors are G protein coupled receptors; therefore, upon S1P binding, many downstream signaling pathways are activated, including PI3K/Akt, Ras/Erk, PLC, Rho, Rac, adenylylate cyclase (16). The intracellular targets of S1P are largely unknown. However a recent study discovered that intracellular S1P inhibits HDAC1/HDAC2 activity thereby regulates histone acetylation and gene transcription (19).

S1P is synthesized by phosphorylation of sphingosine via sphingosine kinases (SphK). There are two sphingosine kinase isoforms, SphK1 and SphK2 (20-22). SphK1 has been shown to
have a role in insulin signaling and in maintaining glucose homeostasis. Specifically, overexpression of SphK1 in KK/Ay diabetic mice lead to decreases in blood glucose levels (23). In addition, high glucose conditions stimulate SphK1 activity in endothelial cells (24). In support of the idea that S1P may be involved in promoting beta-cell viability, SphK activity is increased in the presence of cytokines in INS-1 cells and mouse pancreatic islets (14). Furthermore, of the two isoforms, SphK2 appears to be the dominant active isoform in pancreatic beta cells (25). There are two isoforms of sphingosine 1-phosphate phosphatases, Sgpp1 and Sgpp2, that dephosphorylate S1P to sphingosine (20). To date, neither isoform has been directly implicated in contributing to diabetes.

In this study, we investigated the role of S1P in pancreatic beta-cell function and discovered that exposure to high glucose increases S1P levels by activation of sphingosine kinase isoform 2. Furthermore, we demonstrate that the increase in S1P level is important for glucose–stimulated insulin secretion.

**EXPERIMENTAL PROCEDURES**

**Animals**-C57BL6 mice were purchased from The Jackson Laboratory (Bar Harbor, Maine). Mice were housed at the University of Kentucky Animal Facility. All experiments carried out with animals were performed according to protocols approved by the University of Kentucky’s Institutional Animal Care and Use Committee (IACUC).

**Cell Culture**-Mouse insulinoma-6 (MIN6) cell line was maintained as described previously (26). Experiments were performed using MIN6 cells grown to passages 22-30.

**Insulin Enzyme Linked Immunosorbent Assay (ELISA)**-ELISA was carried out according to the manufacturers’ specifications (Mercodia Inc.). All insulin secretion assays were run in duplicates and results were expressed as fold differences (1mM glucose control=1-fold) in secreted insulin. Insulin levels were normalized to total protein.

**Glucose Tolerance Test**- Mice were IP injected with DMSO or SKI (65mg/kg), followed by a 4 hr starvation period. After starvation, 2g glucose per kg BW was IP injected. Blood glucose (mg/dl) was measured every 15 min up to 120 min. Blood samples were collected to measure insulin levels using the insulin ELISA.

**Insulin Secretion Assay**- Cells were washed with 1x PBS and pre-incubated on DMEM with 1mM glucose for 16hrs. Following this incubation, cells were incubated with freshly made KRBB containing 1mM glucose for 2hrs. For experiments performed with sphingosine kinase inhibitor (SKI, Calbiochem), cells were incubated with 1mM glucose in KRBB for 1.5 hrs, followed by a 30-minute incubation on KRBB with 1mM glucose with or without 10µM SKI or DMSO. Following pre-incubations, cells were incubated in KRBB with 1mM or 25mM glucose for 1hr, at the end of which supernatant was collected and insulin was quantified using ELISA. Insulin secretion in purified islets was assayed by pre-treatment of 50 purified islets with KRBB buffer containing 3mM glucose with or without 40µM SKI or DMSO for 1h. After the pre-treatment, islets were transferred into KRBB buffer containing 3mM or 20mM glucose with or without SKI (40uM) for 1hr and the amount of secreted insulin was quantified using the insulin ELISA assay.

**In vitro Spingosine Kinase Assay**- The sphingosine kinase assay is a modification of a previously described assay (27) and was carried out using the assay buffer (50mM HEPES-pH 7.4, 150mM NaCl, 5mM MgCl2, 1mM DTT, and 3µM sodium orthovanadate) with 1mM ATP, 20µM C17Sphingosine, 10µM C18S1P (internal standard) and cell lysate at 37°C for the indicated times. The reaction was stopped by adding 0.4ml 0.1M HCl. Lipids were extracted and quantified using LC-MS/MS.

**Lipid Extraction and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)**-Sphingolipids were extracted and quantified as previously described (28). Extracted lipids were quantified using the ABI 4000 Q-Trap Mass Spectrometer. The obtained data were normalized to lipid recovery, using the C17 S1P control, and to total starting material, using lipid PO4.

**Pancreatic Islet Isolation**- Pancreatic islets were isolated from C57BL6 mice. Pancreata were removed aseptically via bile duct injection of HBSS (Gibco) containing 0.5mg/ml collagenase type V (Sigma) and incubated at 37°C. Collagenase digestion was stopped by adding chilled HBSS with 10% fetal calf serum. Islets were purified using a histopaque gradient.
The purified islets were incubated in RPMI-1640 with 10% FBS. Islets were handpicked into KRBB and transferred to low (3 mM) or high (20 mM) glucose in KRBB for the indicated time points.

**siRNA Knockdown** - siRNA knockdown experiments were performed as previously described (29, 30). siRNAs were transfected into MIN6 cells using the Amoxa cell line nucleofector kit V (Lonza) according to manufacturer’s specifications. About 4ug siRNA per 1.0 x 10^6 cells was used to achieve optimal knockdown. The sequences of the siRNAs are listed in Supplemental Table 1.

**Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)** - Quantitative Real-Time PCR was performed as previously described (29,30). qRT-PCR was carried out using the Mx4000 machine (Stratagene) with primers for Spp1 (5’ TGGGAGCCATTTCTAGTTG 3’ and 5’ AGCAAACTTCCCCAAACT 3’), pre-pro-insulin (5’ GGGGAGCGTGCTTTTCTCTA 3’ and 5’ GGGACAGAATTTCAGTGGA 3’), and β-actin (5’ CGTGGGCCGCCCTAGGCAACC 3’ and 5’ TTGGCCTTAGGGTTCAGGGG 3’). For Sphk1 (Mm00448841_g1) and β-actin, Taqman probes were used.

**Western Blotting** - Total lysate was prepared from MIN6 cells with standard lysis buffer (50mM Tris-HCl, pH 7.5, 300mM NaCl, 0.1%NP-40, 5mM EDTA and protease/phosphatase inhibitors). Western blot analysis of whole cell lysates was conducted as previously described [29, 30]. Antibodies used are: SphK1 (Santa Cruz, sc-48825) and SphK2 (Santa Cruz, sc-22704).

**Statistical Analysis** - The student-paired t-test was used to determine statistical significance. All experiments analyzed for significance were performed at minimum three times. A p-value < 0.05 was considered statistically significant. Error bars represent +/- standard deviation.

**RESULTS**

**SIP levels are increased under high glucose conditions in pancreatic beta cells** - Sphingolipid levels are known to be altered in obesity and during type 2 diabetes, indicating that these lipids may be involved in regulating glucose homeostasis. Since the production and secretion of insulin in pancreatic beta cells is regulated by changes in glucose levels, we measured sphingosine 1-phosphate (S1P) levels in the mouse insulinoma cell line MIN6 after incubation with 1mM or 25mM glucose. Exposure of MIN6 cells to high glucose (25mM) for 1hr resulted in about 2-fold increase in S1P levels compared to 1mM glucose incubated cells (Fig. 1A), while there were no significant changes in the levels of other sphingolipids, such as sphingomyelin and ceramide (Supplemental Fig. S1). We observed a similar increase in S1P levels in purified mouse pancreatic islets by treatment with 20mM versus 3mM glucose (Fig. 1B). These data suggest that S1P levels are regulated by changes in glucose levels in pancreatic beta cells.

**High Glucose Stimulates SphK Activity in MIN6 cells** - To determine the mechanism(s) by which glucose mediates changes in S1P levels we analyzed SphK expression, localization, and activity. Incubation of MIN6 cells with 1mM or 25mM glucose did not alter SphK1 and SphK2 expression, protein stability or localization (Supplementary Figures S 2 & S3). However, we found that SphK activity was increased over 4-fold in MIN6 cells incubated with high glucose (Fig. 2). Previous data indicate that phorbol 12-myristate 13-acetate (PMA) stimulates SphK activity in other cell types (32). Incubation of MIN6 cells with 1mM glucose in the presence of 100nM PMA stimulated SphK activity by 8-fold compared to 1mM glucose alone (Fig. 2). SphK activity in cell lysates was measured by quantification of newly formed S1P (C17S1P). The SphK activity assay was not linear, which is likely due to the low level of constitutive degradation of S1P by S1P phosphatases. In summary, exposure of MIN6 cells to high glucose stimulates SphK activity in pancreatic beta cells.

**SIP levels are important for GSIS** - Since SphK activity is induced by high glucose and S1P levels are significantly increased in the presence of high glucose, we tested whether changes in S1P levels are important for glucose-stimulated insulin secretion (GSIS). First, we blocked the production of S1P using the sphingosine kinase inhibitor (SKI) (Fig. 3C). MIN6 cells treated with 10µM SKI displayed a significant drop in insulin secretion on 25mM glucose and completely lacked GSIS compared to untreated cells (Fig. 3A). Treatment of MIN6 cells with SKI did not affect total insulin levels (Supplemental Fig. S4). Furthermore, treatment of isolated islets with
40µM SKI for 1hr significantly diminished GSIS (Fig. 3B). These data suggest that the rise in S1P levels in response to high glucose is important for GSIS. To further substantiate the role of S1P in GSIS in pancreatic beta cells, we either blocked S1P production on high glucose by knockdown of SphKs or increased S1P levels on low glucose by knockdown of Sgpp1. Knockdown of both SphK1 and SphK2 by transfection of MIN6 cells with the corresponding siRNA oligos, completely abolished GSIS (Fig. 4A). Surprisingly, knockdown of SphK1 alone did not have a significant effect on insulin secretion (Fig 4B), although knockdown of SphK1 reduced overall S1P levels by 30% (Fig. 4D). However knockdown of SphK2 in MIN6 cells using four different siRNA oligos reproducibly resulted in complete lack of GSIS compared to cells transfected with control siRNA (Fig. 4C). Knockdown of SphK2 resulted in a 75% decrease of S1P levels (Fig. 4D). Thus, knockdown of SphK2 completely abolished GSIS. The efficiency of SphK1 and SphK2 knockdown in MIN6 cells was confirmed as shown in Supplemental Fig. S5. Knockdown of SphK2 did not affect cell viability compared to cells transfected with control siRNA (Supplemental Fig. S6). In support of an important role for Sphk2 in beta cells, glucose-induction of Sphk activity was reduced by 6-fold in MIN6 cells transfected with SphK2 siRNAs compared to control cells (Fig. 5). These data suggest that the increase in S1P levels by high glucose is likely due to activation of SphK2 in the presence of glucose.

Consistent with a role for S1P in GSIS, knockdown of Sgpp1 did not have any effect on insulin secretion on high glucose, but increased insulin secretion by about 3-fold on low glucose (Fig. 6A). Sgpp1 dephosphorylates S1P to sphingosine and thereby reducing S1P levels in the cells. Knockdown of Sgpp1 resulted in about 50% increase of S1P levels (Fig. 6B) that lead to an increase in insulin secretion (Fig. 6A). The efficiency of Sgpp1 knockdown using four different siRNA oligos was confirmed as illustrated in Supplementary Fig. S7.

**IP-Injection of SKI into mice impairs glucose disposal**- Since SKI inhibits GSIS in MIN6 cells, we tested whether SKI treatment of mice affected insulin secretion and thereby glucose disposal during the glucose tolerance test (IP-GTT). For this purpose eight week old C57BL6 mice were IP-injected with DMSO (control mice) or with 65m/kg BW SKI and starved for 4hrs. Following the starvation period, the mice were IP-injected with 2g/kgBW glucose and blood glucose and insulin levels were determined (Fig. 7). Within 30min after IP-injection of glucose, the SKI treated mice had a significant higher blood glucose levels than control mice (Fig. 7A&B).

The glucose intolerance observed in SKI treated mice correlated with decreased serum insulin levels compared to control mice (Fig. 7C&D). Plasma insulin levels in SKI treated mice were significantly lower than in DMSO treated control mice (Fig. 7C). The IP-GTT experiments suggest that treatment of mice with the sphingosine kinase inhibitor SKI inhibits insulin secretion and thereby results in impaired glucose disposal.

**DISCUSSION**

In this study, we have presented evidence that sphingosine kinase activity is regulated by glucose in pancreatic beta cells. Exposure of beta cells to high glucose induces SphK activity, leading to a rise in S1P levels. The increase in S1P levels during high glucose conditions is important for GSIS. Inhibition of S1P production by treatment of cells with the sphingosine kinase inhibitor SKI or by knockdown of SphK2 using siRNAs caused a complete lack of GSIS in pancreatic beta cells. On the other hand, knockdown of Sgpp1, that normally dephosphorylates S1P, led to increased insulin secretion even on low glucose. We conclude from these data that S1P has a novel role in pancreatic beta cells and that changes in S1P levels are important for GSIS.

Although it has previously been reported that exogenously administered S1P to HIT-T15 beta cells stimulates insulin secretion, it was not clear from this previous study whether addition of exogenous S1P mimicked GSIS (15). Extracellular S1P can function as a signaling molecule by binding to five G-protein coupled S1P receptors, but intracellular S1P can also activate distinct signal pathways (33, 34). We believe that the regulation of GSIS by S1P in pancreatic beta cells involves the activation of intracellular targets of S1P, because the rise in S1P coincides with GSIS. Furthermore, we could not
detect any extracellular S1P in the media of MIN6 cells using LC-MS/MS (data not shown).

Regulation of SphK activity and S1P levels by glucose has also been reported previously in endothelial cells (24), suggesting that S1P may play a key role in maintaining glucose homeostasis. Consistent with our data, a recent report also demonstrates that SphK activity is regulated by high glucose in rat pancreatic islets (35).

Knockdown of SphK1 in MIN6 cells had no effect on GSIS. This is interesting considering the fact that knockdown of SphK1 reduced S1P levels by 30% without having an effect on GSIS. It is possible that the overall increase in S1P levels is not important for GSIS, but increases in S1P levels at specific locations or compartments, such as the granules may be required for GSIS. Knockdown of SphK2 led to a 75% decrease in S1P levels and abolished GSIS in MIN6 cells. This suggests that the observed increase in SphK activity mediated by high glucose is due to activation of SphK2 and that the rise in S1P levels by glucose is likely to be mediated by activation of SphK2. Indeed, knockdown of SphK2 drastically reduced the glucose-induced SphK activity in MIN6 cells (Fig. 5), confirming that the rise in S1P levels on high glucose is mainly due to SphK2 activity. Consistent with this idea, a previous study reported that SphK2 is the major active isoform in pancreatic beta cell lines and rat islets (25).

Although the mechanism(s) by which glucose activates SphK2 in pancreatic beta cells remains to be established, it is most likely that glucose stimulates SphK activity by a post-translational modification. It has been previously shown that PMA induces the phosphorylation and activation of both SphK1 and SphK2 by stimulating the activation of protein kinase C (PKC) and extracellular signal-related kinases 1/2 (ERK1/2) (36-38). These same signaling pathways are also important for glucose-induced β-cell function. Our data show that PMA induces SphK activity on low glucose conditions, and that this increase in activity is comparable to that seen on high glucose.

Glucose tolerance experiments in mice indicate that IP-injection of SKI impairs glucose disposal in mice due to inhibition of insulin secretion. These data suggest that S1P has an important role in GSIS in rodents. While deletion of SphK1 and SphK2 in mice causes lethality, SphK1 or SphK2 single knock-out mice are viable (39, 40). Neither the SphK1 nor the SphK2 knock-out mice have been reported to be diabetic. Our data presented here suggest that SphK2 activity is essential for GSIS in the MIN6 cell line. Thus, it is expected that mice deleted for SphK2 should have defects in insulin secretion. One explanation why SphK2 knockout mice are not diabetic could be that SphK1 compensates for the lack of SphK2. Alternatively, other compensation mechanisms could be active in SphK2 knock out mice, such as decreased S1P phosphatase activity. However, detailed studies on insulin secretion and glucose disposal in SphK2 knock out mice are lacking.

While S1P levels increased during incubation with high glucose within the pancreatic beta cells, the levels of other sphingolipids, including sphingomyelin and ceramide were not changed. A large body of evidence indicates that chronic hyperglycemia and hyperlipidemia caused by nutrient excess and diabetes, results in dramatic increases in ceramide levels (1,5-7,9). Ceramide and S1P are known to have opposing actions. Typically, when ceramide levels are increased, S1P levels are decreased and vice versa. Therefore, it is likely that under normal conditions S1P has an important function in pancreatic beta cells and regulates GSIS, but under diabetic conditions, increases in ceramide levels may oppose the actions of S1P, contributing to defects in insulin secretion. Since ceramide appears to negatively affect glucose-stimulated insulin gene transcription, it is possible it may have the same affect on insulin secretion during diabetes (11). Thus, it would be interesting to determine if S1P levels are altered within pancreatic islets of diabetic animals, and if so, does activation of SphK2 within diabetic islets restore impaired GSIS.

In summary, consistent with previous data that indicate an important role for sphingolipids in glucose homeostasis, we demonstrate in this report that changes in S1P levels in pancreatic beta cells impact GSIS. Furthermore, we show that changes in S1P levels are mediated by regulation of SphK2 activity by glucose. Thus, altered S1P levels may interfere with normal glucose homeostasis and contribute to metabolic disorders such as diabetes. Detailed studies on S1P function in pancreatic beta
cells may contribute to the development of novel strategies for the treatment of diabetes.

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FOOTNOTES

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The abbreviations used are: S1P, sphingosine 1-phosphate; SphK, sphingosine kinase; GSIS, glucose-stimulated insulin secretion; MIN6, mouse insulinoma 6; SKI, sphingosine kinase inhibitor; Sgpp, sphingosine 1-phosphate phosphatase.

FIGURE LEGENDS

Fig. 1. S1P levels are increased under high glucose conditions. (A) S1P levels were quantified in MIN6 cells after incubation in KRBB containing 1mM or 25mM glucose for 1 hr. (B) S1P levels were quantified in mouse pancreatic islets after incubation in KRBB containing 3mM or 20mM glucose for 1 hr. After extraction of lipids from MIN6 cells or islets, S1P levels were quantified using LC-MS/MS. Values were normalized to C17 S1P and total lipid PO4. The results are mean values of three independent experiments (n = 3) ± SEM. * p < 0.01 versus 1mM glucose.

Fig. 2. Glucose stimulates sphingosine kinase activity in MIN6 cells. Lipids were extracted from MIN6 cells incubated with KRBB containing 1mM, 25mM glucose or 1mM glucose with 100nM PMA for 1 hr. SphK activity was measured at various time points (0, 10, and 30 min) by quantification of C17-S1P formation by LC-MS/MS.

Fig. 3. Inhibition of sphingosine kinase activity decreases GSIS. Insulin secretion from MIN6 cells incubated with KRBB containing 1mM or 25mM glucose +/- SKI for 1hr was measured using insulin ELISA as described in detail in the Methods section (A). Insulin secretion in pancreatic islets was measured by incubating islets with 3mM or 20mM glucose +/- SKI for 1hr (B). Values are normalized to
total insulin and expressed as fold differences, where 1mM glucose control (-SKI) was set to 1 (n = 5) ± SEM. * p < 0.05 versus 25mM glucose control (-SKI). (C) S1P levels were quantified in MIN6 cells treated with KRBB containing 1mM glucose, 25mM glucose, or 1mM glucose plus PMA with or without SKI for 1hr using LC-MS/MS. Values are normalized to total lipid PO4 (n=5). * p < 0.05 versus 1mM, 25mM glucose control (-SKI) or 1mM glucose +PMA (-SKI).

Fig. 4. Knockdown of SphK2 in MIN6 cells abolishes GSIS. siRNA knockdown was carried out in MIN6 cells using siRNA oligos, along with control siRNA, targeting both SphK1 and SphK2 (A), only SphK1 (B) and only SphK2 (C). Insulin secretion was assayed in the media after treatment with 1mM or 25mM glucose for 1hr. Values are normalized to total protein and are expressed as fold differences, with 1mM glucose control set as 1 (A, n=3; B, n=3; C, n=4) ± SEM. * p < 0.01 verses 25mM glucose control. (D) S1P levels were quantified in MIN6 cells transfected with siRandom, siSphK1 or siSphhK2 oligonucleotides and incubated on 25mM glucose. Values are normalized to total lipid PO4 (n=2).

Fig. 5. Knockdown of SphK2 abolishes the glucose-induced SphK activity. MIN6 cells were transfected with four different SphK2 siRNA or control siRNA oligos. After incubation for 48 hrs, cells were treated with KRBB containing 25mM glucose for 1hr and protein extracts prepared. SphK activity was measured at various time points (0, 10, and 30 min) by quantification of C17-S1P formation using LC-MS/MS (n = 3) ± SEM. * p < 0.05 verses siRandom.

Fig. 6. Knockdown of S1P phosphatase Sgpp1 increases insulin secretion on low glucose. (A) siRNA knockdown was carried out in MIN6 cells using four different siRNA oligos targeting Sgpp1 along with control siRNA,. Insulin secretion was measured in the media using insulin ELISA and normalized to total protein. Values are expressed as fold differences, setting 1mM glucose control as 1 (n=8) ± SEM. (B) S1P levels were quantified in MIN6 cells transfected with siRandom or siSgpp1 oligonucleotides and incubated on 1mM glucose. Values are normalized to total lipid PO4 (n=2).

Fig. 7. Mice treated with the sphingosine kinase inhibitor SKI have impaired glucose tolerance and decreased plasma insulin levels. 10 weeks old C57BL6 mice were injected with DMSO (control) or 65mg/kgBW SKI and starved for 4 hrs. After starvation, the mice were IP injected with 2g/kgBW with glucose (IP-GTT) and blood glucose (panel A&B, Control n=6; SKI treated mice n=5) and plasma insulin (panel C&D, n=4) levels were monitored every 15 min and quantified using insulin ELISA. (*p<0.05).
Figure 1

A

B

S1P (pmol)/Lipid PO₄ (nmol)

0

0.1

0.2

0.3

1mM 25mM

S1P (pmol)/Lipid PO₄ (nmol)

3mM 20mM
Figure 2
Figure 3

A

MIN6

B

Pancreatic Islets

C

Fold Difference in Secreted Insulin

Fold Difference in S1P Levels

- SKI  + SKI

1mM  25mM

1mM  25mM

3mM  20mM

- SKI  + SKI

3mM  20mM

1mM + PMA

*
Figure 5

[Graph showing the change in C_{17-}S1P (pmol)/Total Protein (mg) over time for siRandom and siSphK2 treatments.]
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