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Stevioside counteracts the glyburide-induced desensitization of the pancreatic beta-cell function in mice: studies in vitro

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Abstract

The sulfonylurea glyburide (GB) is one of the most frequently used drugs in diabetes treatment. Long-term pretreatment with GB causes elevated basal insulin secretion (BIS) and decreased glucose-stimulated insulin secretion (GSIS). These characteristics may play an important role for the development of hypoglycemia and secondary failure. Stevioside (SVS), a substance extracted from leaves of *Stevia rebaudiana* Bertoni, enhances GSIS but not BIS. The aim of the present study was to clarify whether 24-hour exposure of isolated mouse islets to GB causes dose-dependent decrease in the GSIS and whether it is possible to counteract this desensitization by SVS. We also tested the impact of the incretin glucagon-like peptide-1 (GLP-1) on the GB-induced desensitization. After 24-hour preincubation with GB in combination with SVS or GLP-1, we measured the basal and glucose-stimulated insulin responses and the total islet insulin content. We also determined the fold change in gene expression of pancreatic and duodenal homeobox 1 and glucose transporter isoform 2. After 24-hour preincubation in 11.1 mmol/L glucose, GB (10^{-11}-10^{-3} mol/L) caused a dose-dependent decrease in GSIS (16.7 mmol/L glucose) ($P < .001$). GB (10^{-7} mol/L) pretreatment elevated BIS, but neither SVS (10^{-7} mol/L) nor GLP-1 (10^{-7} mol/L) could reverse this. Interestingly, the GB-induced desensitization of GSIS was counteracted by both SVS ($P < .05$) and GLP-1 ($P < .05$). SVS reversed the decrease in insulin content caused by GB pretreatment ($P < .05$). GB pretreatment did not change gene expression of pancreatic and duodenal homeobox 1 nor glucose transporter isoform 2, whereas SVS significantly up-regulated the expression of both genes by more than 2-fold ($P < .05$). Our results showed that SVS in combination with GB did not reverse GB-induced increase in BIS, whereas both SVS and GLP-1 counteracted GB-induced desensitization of GSIS. SVS is able to counteract the desensitizing effects of GB and may be a putative new drug candidate for the treatment of type 2 diabetes mellitus.

1. Introduction

Insulin resistance and beta-cell dysfunction are fundamental defects that contribute to the development of type 2 diabetes mellitus (T2DM), and as such are targets for primary prevention of disease progression. The United Kingdom Prospective Diabetes Study (UKPDS) has demonstrated that in T2DM, complications may be significantly delayed or even prevented with strict glycemic control [1,2]. T2DM typically becomes more severe and difficult to treat over time. Thus, most patients require drug therapy with antihyperglycemic agents soon after the disease is diagnosed. Sulfonylureas are widely used for the treatment of T2DM. They enhance insulin release from beta cells by blocking the adenosine triphosphate (ATP)-dependent K^+ channels and subsequently stimulate exocytosis of the insulin-containing granules [3]. However, long-term use of these oral hypoglycemic agents induces desensitization to metabolic stimuli in patients with T2DM [4]. In normoglycemic rats, long-term treatment with sulfonylureas results in lower insulin content and reduced insulin responsiveness [5-7]. In the clinical setting, diabetic subjects often develop secondary failure after long-term treatment with sulfonylureas [8]. Thus, UKPDS showed that monotherapy with oral agents often fails to maintain glycemic control over time, and many patients have to switch to treatment with combinations of oral agents or insulin therapy [1,2].

Although there are many putative causes underlying this secondary failure, sulfonylurea treatment per se may contribute to this so-called desensitization [9,10]. Recently, it has been shown that a reduction in the amount of pancreas duodenum homeobox 1 (PDX-1) impairs glucose-stimulated insulin secretion (GSIS) [11]. PDX-1 is a

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transcription factor that activates glucose transporter type 2 (GLUT2) gene transcription [11]. The regulation of PDX-1 and GLUT2 gene is of central importance for glucose responsiveness of the pancreatic beta cells. In addition, PDX-1 has been implicated in the development of the pancreas, which includes maturation and differentiation of pancreatic precursor cells. Mutations in the PDX-1 gene have been shown to cause pancreatic agenesis and is implicated in maturity-onset diabetes of young subjects [12]. Although Tiedge and Lenzen [13] did not find any change in GLUT2 in pancreatic beta cells from rats treated with glyburide (GB), one of the most frequently used sulfonylureas, it is not clear whether these genes are to some extent involved in the GB-induced desensitization.

Stevioside (SVS), a diterpene glycoside, is one of the main extracts from the leaves of the plant *Stevia rebaudiana* Bertoni. It possesses insulinotropic, glucagonostatic, antihyperglycemic, and blood-pressure-lowering effects [14-19]. SVS does not, like sulfonylureas, close ATP-sensitive potassium channels in beta cells [19]. Interestingly, it increases proinsulin content at both messenger RNA and protein levels [18]. SVS shares important characteristics with glucagon-like peptide-1 (GLP-1) on islet cell function [20]. We found that the basal insulin secretion (BIS) did not change after short-term pretreatment with SVS, whereas BIS increased about 3-fold after pretreatment with GB. GSIS increased dose-dependently after 24-hour pretreatment with SVS as well as GLP-1, but decreased after pretreatment with GB [20]. However, we did not explore whether SVS or GLP has the potential to counteract the GB-induced desensitization [20]. Because sulfonylureas are widely used based on their positive effects on hyperglycemia in T2DM, it is of interest to clarify whether the desensitization can be reversed. Consequently, we investigated whether SVS and GLP-1 may possess positive effects on the GB-induced desensitization in isolated mouse islets.

2. Materials and methods

2.1. Experimental animals

Adult female NMRI mice (Bomholtgaard Breeding and Research Centre, Ry, Denmark) weighing 20 to 25 g were used. The animals were kept on a standard pellet diet, tap water ad libitum, and a light/dark cycle of 12 hours.

This study was carried out in accordance with the guidelines of the Danish Council on Animal Care.

2.2. Islet isolation

Islets were isolated by the collagenase digestion technique [21] as previously described by us [20]. In brief, the pancreas was retrogradely filled with 3 mL ice-cold Hanks balanced salt solution (Sigma Chemical, St Louis, MO) supplemented with 0.3 mg/mL collagenase P (Boehringer Mannheim, Mannheim, Germany). The pancreas was removed, incubated for 19 minutes at 37°C in a water bath, and subsequently rinsed with ice-cold Hanks balanced salt solution, and the islets were handpicked under a stereomicroscope. Very large and very small islets were discarded and only medium-sized islets were used for further studies. The islets were then incubated overnight at 37°C and 95% normal atmosphere/5% carbon dioxide in 10 mL RPMI 1640 containing 11.1 mmol/L glucose. All RPMI used in this article contained 10% fetal calf serum. Islets for the incubation studies were obtained from 6 to 10 mice to compensate for interindividual differences.

2.3. Agent preparation

GB (Sigma) was added to the medium from stock solutions (10^{-1}, 10^{-3}, 10^{-5}, 10^{-7}, or 10^{-9} mol/L) in dimethyl sulfoxide (DMSO) (Merck, Frankfurt, Germany); the final concentration of DMSO in all the media of the GB dose-dependent study was 1%, and in the rest of the studies was 0.01%. SVS (Wako Pure Chemical Industries, Osaka, Japan) was added to the medium from a stock solution (10^{-3} mol/L) in distilled water, and GLP-1 (Sigma, St Louis, MO) from a stock solution (10^{-4} mol/L) in distilled water.

2.4. Islet incubation and insulin secretion studies

The first part of the study was designed to determine the dose-response of GB-induced desensitization. After overnight incubation, mouse islets were preincubated for 24 hours in RPMI (containing 10% fetal calf serum) with 11.1 mmol/L glucose and GB at concentrations of 0, 10^{-11}, 10^{-9}, 10^{-7}, 10^{-5}, or 10^{-3} mol/L. In the second part of the study, after overnight incubation, mouse islets were preincubated for 24 hours in RPMI with the addition of 10^{-7} mol/L GB, 10^{-7} mol/L GB plus 10^{-7} mol/L SVS, or 10^{-7} mol/L GB plus 10^{-7} mol/L GLP-1, respectively.

After the above-mentioned different preincubations, islets were rinsed once with modified Krebs-Ringer buffer (KRB) supplemented with 3.3 mmol/L glucose and 0.1% human serum albumin (Sigma). The KRB contained 125 mmol/L NaCl, 5.9 mmol/L KCl, 1.2 mmol/L $MgCl_2$, 1.28 mmol/L $CaCl_2$, and 25 mmol/L HEPES (pH 7.4, all from Sigma). After 30-minute preincubation in normal atmosphere at 37°C, single islets were handpicked and incubated in 100 μL KRB with glucose concentration of 3.3 or 16.7 mmol/L. Our experiments were designed to pair the size of islets in different groups to balance the diversity of islet size. A similar distribution of islet size was used in different groups. After 60-minute incubation in normal atmosphere at 37°C, 50 μL of the medium was collected and frozen for analysis of insulin.

2.5. Insulin content

After 24-hour culture with the different agents as described in the second part of the study, groups of 10 islets each were transferred to 1 mL of glycine-bovine serum albumin (BSA) (Roche Molecular Biochemicals, Mannheim, Germany) buffer (glycine 100 mmol/L, 0.25%

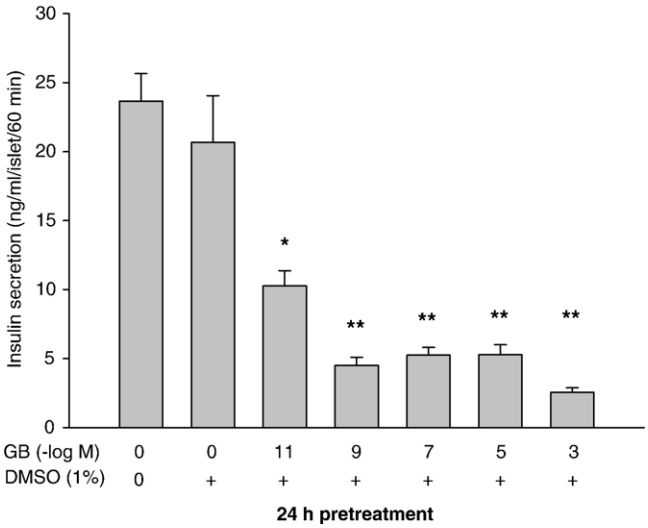

Fig. 1. Dose-dependent effects of GB pretreatment on GSIS in isolated mouse islets. After 24-hour pretreatment with GB ranging from 0, 10^{-11}, 10^{-9}, 10^{-7}, 10^{-5}, and 10^{-3} mol/L in RPMI with 11.1 mmol/L glucose and 1% dimethyl sulfoxide (DMSO), single islets were incubated at 16.7 mmol/L glucose for 1 hour and the medium was collected for insulin assay. Each bar presents the mean ± SEM from 16 incubations of single islets. $^{*}P < .05$ and $^{**}P < .001$ denote significant differences from the control without GB.

BSA, pH 8.8). Insulin was released by sonicating twice (Branson Sonifier 250, Danbury, CT) for 14 seconds on ice. After centrifugation for 30 minutes at 16000 rpm, the supernatant was collected and frozen at −20°C for later insulin assay.

2.6. Insulin assay

Insulin was analyzed by radioimmunoassay using a guinea pig antiporcine insulin antibody (Novo Nordisk, Bagsvaerd, Denmark) and mono-[^{125}I]Tyr A14–labeled human insulin (Novo Nordisk) as tracer and rat insulin as standard (Novo Nordisk). The separation of bound and free radioactivity was performed using ethanol [22]. The inter- and intra-assay variation coefficients were both less than 10%. SVS, GB, and GLP-1 did not interfere with the insulin assay at the concentrations studied.

2.7. Isolation of RNA

After 24-hour culture with the different agents as described in the second part of the study (except 10^{-7} mol/L GB plus 10^{-7} mol/L GLP-1), 200 islets were transferred to 1 mL TriZol reagent (Gibco BRL Life Technologies, Roskilde, Denmark) and total RNA was extracted according to the manufacturer's instructions. RNA was quantified by measuring absorbency at 260 and 280 nm. The integrity of the RNA was checked by visual inspection of the 2 ribosomal RNAs 18S and 28S on an agarose gel.

2.8. Real-time reverse transcription-polymerase chain reaction

Relative real-time transcription-polymerase chain reaction (PCR) was performed on an ICycler machine (BioRad) using SYBR-Green detection and 18S rRNA as internal normalizer. Complementary DNA was made by using IScript (BioRad). The RNA samples were treated with deoxyribonuclease to remove residual genomic DNA (TURBO DNA-free, Ambion, Austin, TX). Primer pairs were designed by using OLIGO 6.0 (Medprobe, Oslo, Norway). The primer efficiency was optimized with temperature gradient PCR for different primer concentrations, and specificity was evaluated by using melt curves and standard electrophoresis. All primer pairs were optimized to amplify the amplicon to near-100% efficiency, and therefore we used the ΔΔCt method to calculate the relative gene expression (as described in User Bulletin 2, 1997, from Perkin-Elmer covering the aspect of relative quantization of gene expression). All samples were amplified in triplicate. No template controls and no amplification controls were included for each gene as negative controls.

PCR cycling parameters are as follows: 95°C for 3 minutes, 45 cycles (95°C for 30 seconds, 56°C to 60°C for 20 seconds, 72°C for 20 seconds), 72°C for 10 minutes. Primers used were PDX-1 sense 5′-CCTCCACCACCACCTTCCA-3′, antisense 5′-CGCTGTGTAAGCACCTCCTG-3′; GLUT2 sense 5′-GGAAGGATCAAAGCAATGTTG-3′, antisense 5′-TTCGTCCAGCAATGATGAG-3′; and 18S rRNA sense 5′-CTTTGGTCGCTCGCTCCTC-3′, antisense 5′-ACCGGGTTGGTTTTGATCTG-3′.

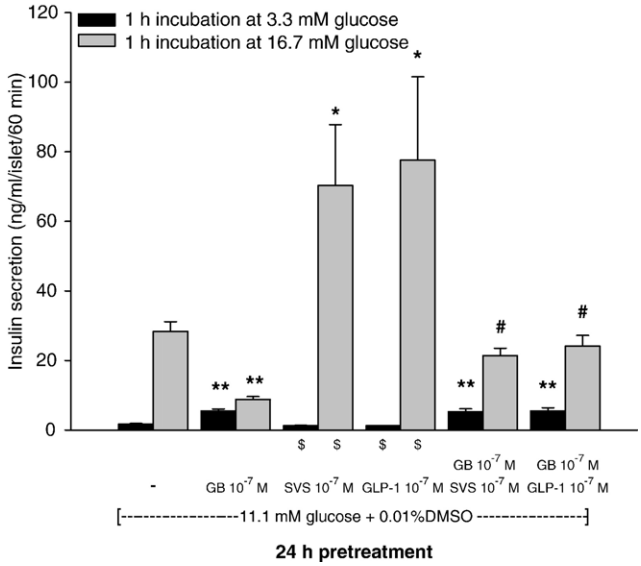

Fig. 2. The effects of SVS or GLP-1 on GB-induced BIS and GSIS in isolated mouse islets. Islets were treated for 24 hours with 10^{-7} mol/L GB alone or in combination with 10^{-7} mol/L SVS or 10^{-7} mol/L GLP-1 in the presence of 11.1 mmol/L glucose and 0.01% DMSO. Single islets were subsequently removed from the medium and incubated in 3.3 or 16.7 mmol/L glucose alone for 1 hour. Each bar represents the mean ± SEM from 32 incubations of single islets. The control data of SVS and GLP-1 alone (marked with "$") have previously been published [20]. $^{*}P < .05$ and $^{**}P < .001$ denote significant differences from the control without GB. $^{\#}P < .05$ denotes significant differences from the control vs that of GB alone.

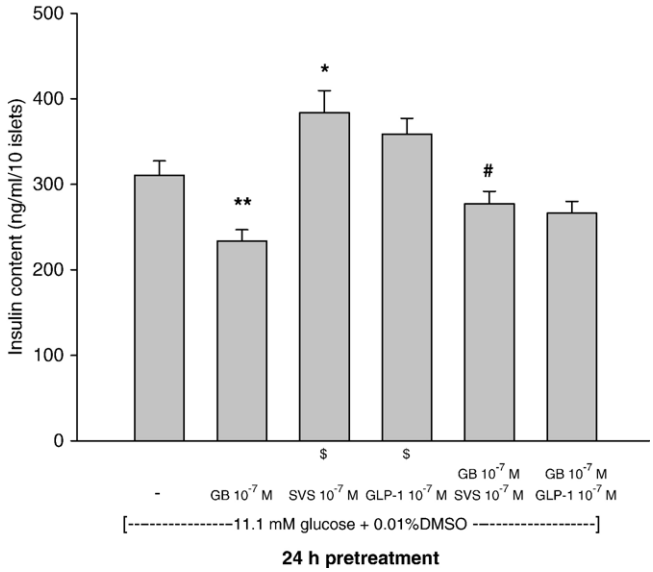

Fig. 3. The effects of GB alone or in combination with SVS or GLP-1 on insulin content in isolated mouse islets. One hundred islets were cultured in each Petri dish for 24 hours with 10^{-7} mol/L GB alone or in combination with 10^{-7} mol/L SVS or 10^{-7} mol/L GLP-1 in the presence of 11.1 mmol/L glucose and 0.01% DMSO. Subsequently, 10 islets each were transferred to 1 mL of glycine-BSA buffer and sonicated after which insulin measurements were carried out. The insulin content was expressed as nanograms insulin per milliliter per 10 islets. Each bar represents the mean ± SEM from 30 repeats. $^{\$}$Indicates the groups of SVS and GLP-1 alone that have previously been published [20]. *P < .05 and **P < .001 denote significant differences from the control without GB. $^{\#}P$ < .05 denotes significant differences from that of GB alone.

2.9. Statistical analysis

We performed statistical analysis with one-way analysis of variance or Student unpaired t test. Each treatment condition was compared with control. We considered differences between 2 groups significant at P values less than .05. Data are expressed as mean ± SEM.

3. Results

3.1. GB-induced dose-dependent desensitization of beta cells

After 24-hour pretreatment with GB at concentrations of 10^{-11}, 10^{-9}, 10^{-7}, 10^{-5}, or 10^{-3} mol/L in the presence of 11.1 mmol/L glucose and 1% DMSO, we exposed the islets for 1 hour to 16.7 mmol/L glucose. We found a significant decrease in the GB-induced GSIS (n = 16, P < .001). As can be seen in Fig. 1, GB decreased GSIS in a dose-dependent manner. Even at the very low level (10^{-11} mol/L), we found a desensitizing effect of GB pretreatment (n = 16, P < .05). DMSO (1%) had no effect on insulin secretion (n = 16, P = 0.46).

3.2. Effects on the BIS and GSIS of pretreatment with GB in the presence of SVS or GLP-1

After 24-hour pretreatment with GB (10^{-7} mol/L) alone or in the presence of SVS (10^{-7} mol/L) or GLP-1 (10^{-7} mol/L), the islets were subsequently incubated for 1 hour with low (3.3 mmol/L) or high (16.7 mmol/L) glucose concentration (Fig. 2). As expected, GB per se increased the BIS at low glucose concentration (321% ± 32.5% vs 100% ± 13.8%, n = 32, P < .001). SVS or GLP-1 in combination with GB did not change the enhanced BIS (307% ± 50.5% or 321% ± 50.2% vs 100% ± 13.8%, n = 32, P < .001, respectively). In accordance with the dose-finding study mentioned above, we found the pretreatment with GB decreased GSIS (16.7 mmol/L glucose) (30.9% ± 3.2% vs 100% ± 9.8%, n = 32, P < .001). SVS and GLP-1 were able to counteract the GB-induced desensitization of GSIS, ie, increased the GSIS after 24-hour pretreatment (75.5% ± 7.5% or 85.4% ± 10.6% vs 30.9% ± 3.2%, n = 32, P < .001, respectively).

3.3. Effects on the insulin content of pretreatment with GB and in the presence of SVS or GLP-1

Islets were pretreated with GB (10^{-7} mol/L) alone or GB (10^{-7} mol/L) with SVS (10^{-7} mol/L) or GLP-1 (10^{-7} mol/L) for 24 hours. Subsequently, 10 islets were sonicated and the insulin content was determined. As seen in Fig. 3, GB significantly decreased insulin content in

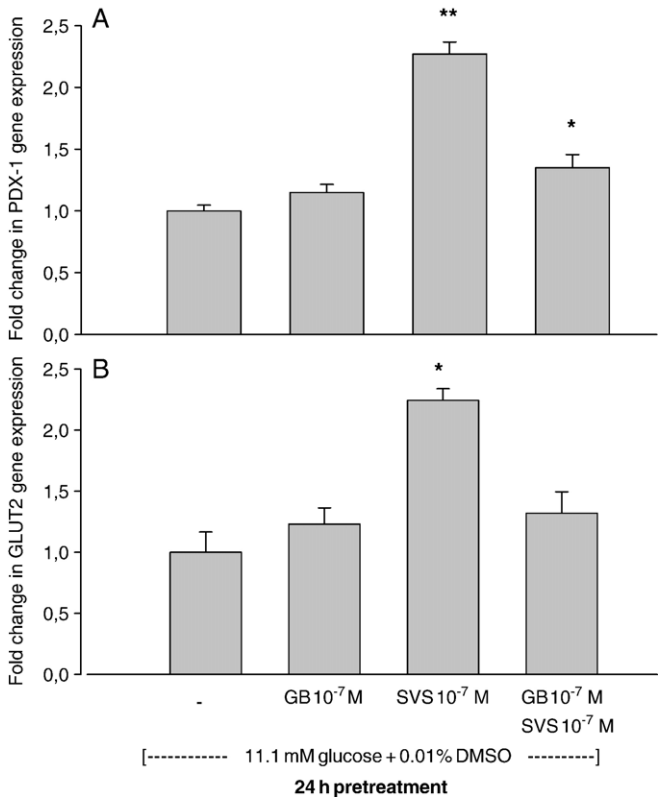

Fig. 4. The effects of GB, SVS, or GB and SVS on (A) PDX-1 or (B) GLUT2 gene expression in isolated mouse islets. After 24-hour pretreatment with 10^{-7}mol/L GB, 10^{-7} mol/L SVS, or 10^{-7} mol/L GB plus 10^{-7} mol/L SVS in the presence of 11.1 mmol/L glucose and 0.01% DMSO, 200 islets were used to extract RNA. All data are expressed as fold change relative to the gene expression of controls (mean ± SEM of 3 separate observations). *P < .05 and **P < .001 denote significant differences from the control without GB.

mouse islets (n = 32, $P < .001$). Only SVS was able to counteract the decreased insulin content in the islets (n = 32, $P < .05$). GLP-1 had a tendency to reverse impaired insulin content, but the P value was not statistically significant (n = 32, $P < .096$).

3.4. Effects on the PDX-1 and GLUT2 gene expression after pretreatment with GB in the presence of SVS

The gene expressions of PDX-1 and GLUT2 were determined after 24-hour pretreatment with GB (10^{-7} mol/L), SVS (10^{-7} mol/L), or the combination (Fig. 4A and B). GB pretreatment did not significantly change PDX-1 or GLUT2 gene expression (n = 3, $P = 0.07$; n = 3, $P = 0.14$, respectively). In contrast, SVS enhanced the gene expression of PDX-1 (230% ± 10% vs 100% ± 5%, n = 3, $P < .001$) and GLUT2 (220% ± 9% vs 100% ± 17%, n = 3, $P < .05$). The combination of SVS and GB significantly increased the gene expression of PDX-1 compared with control (140% ± 10% vs 100% ± 5%, n = 3, $P < .05$), whereas it did not significantly differ from that of GB alone (140% ± 10% vs 110% ± 7%, n = 3, $P = 0.127$) (Fig. 4A). The combination of SVS with GB did not significantly increase GLUT2 expression compared with control (130% ± 17% vs 100% ± 17%, n = 3, $P = 0.17$) or with GB alone (130% ± 17% vs 120% ± 13%, n = 3, $P = 0.67$) (Fig. 4B).

4. Discussion

In the present study, we demonstrated that 24-hour GB pretreatment causes elevated BIS and dose-dependent decrease in GSIS. SVS and GLP-1 in combination with GB cannot counteract the increased BIS but counteracts the GB-induced desensitization of GSIS. A small increase in insulin content accompanies the counteractive effects of SVS. The clinical importance of this finding is unknown. SVS enhances PDX-1 and GLUT2 gene expression more than 2-fold; furthermore, SVS in combination with GB increases PDX-1 gene expression compared with control.

Recently, we have revealed that SVS, a compound isolated from the plant *S rebaudiana* Bertoni, exerts a direct insulinotropic action in isolated mouse islets and the clonal beta-cell line, INS-1, in a glucose-dependent way apparently without affecting KATP-sensitive channels or the cyclic adenosine monophosphate (cAMP) system [18,19,23]. In contrast to the effects of pretreatment with GB, SVS pretreatment does not cause a stimulation of BIS and does not desensitize beta cells [20]. Now we explored the effects of the combination of SVS and GB in terms of increased BIS and decreased GSIS.

Previous studies either on whole pancreas or on isolated islets using dynamic perifusion or static incubation methods showed that GB can cause desensitization [9,24-26]. We chose the static model because it saves time as well as isolated mouse islets. However, the static model is not the best to use to study desensitization in vitro; special caution should be taken in interpreting the data. GB at concentrations ranging from 10^{-11} to 10^{-3} mol/L caused a dose-dependent desensitization. The concentration of 10^{-6} mol/L GB corresponds to the peak concentration in serum 1 or 2 hours after administration of 2.5 mg of GB [27]; the concentration of GB falls to 10^{-8} mol/L after 12 to 24 hours [28]. Consequently, a GB concentration of 10^{-7} mol/L was selected for our further studies. An equimolar concentration of SVS (10^{-7} mol/L) was chosen based on its high potency [19,20]. Elevated levels of proinsulin are considered indicative of beta-cell dysfunction [29]. Previously, we have shown that 24-hour SVS pretreatment does not increase BIS per se [20]. In the present study, we found that neither SVS nor GLP-1 was able to counteract the GB-induced increase in BIS.

Data from UKPDS indicates that 6 years' monotherapy with sulfonylurea is associated with 44% secondary failure [8]. In this regard, desensitization appears to be an important step in the secondary failure of oral antidiabetic treatment [30]. We have previously demonstrated that 24-hour exposure to SVS as well as GLP-1 enhances GSIS [20]. Furthermore, SVS increases insulin content as well, whereas GLP-1 only tends to increase the insulin content [20]. This provided the possibility of exploring whether administration of SVS or GLP-1 can counteract the GB desensitization. In the present study, we demonstrated that SVS as well as GLP-1, to some extent, could counteract GB-induced desensitization in the static model of insulin secretion. According to Bratanova-Tochkova et al [31], there are 4 well-established pathways of beta-cell stimulus-secretion coupling, as follows: (1) the KATP channel–dependent pathway, (2) the KATP channel–independent, Ca^{2+}-dependent pathway of glucose action, (3) activation of phospholipases and protein kinase C (PKC), and (4) stimulation of adenylyl cyclase activity and activation of protein kinase A. An SVS-induced activation of phospholipases and PKC is a possible pathway because no cAMP change is involved in this signal transduction, and we previously demonstrated that SVS does not affect the cAMP levels [19]. This pathway increases phosphoinositide turnover resulting in mobilization of stored calcium to increase $[Ca^{2+}]i$ level and increasing production of diacylglycerol, which activates PKC isoforms.

The counteractive effect of SVS on GB desensitization was accompanied by a small increase in insulin content. GLP-1 tended to increase the insulin content, but did not reach statistical significance. There was no statistically significant difference between the effects of SVS and GLP-1 on the GB-induced effects on insulin content.

It should be noted that we have added only 0.01% DMSO to the media. Kemp and Habener [32] recently reported that 0.5% to 2.5% DMSO had a synergistic effect on GLP-1–stimulated insulin secretion and gene transcription in INS-1 cells. However, the concentration of DMSO we used is more than fifty times less. Thus, we find it unlikely that the stimulatory effect of GLP-1 on GSIS can be explained by an action of DMSO, but more likely an effect of GLP-1 per se.

SVS enhanced PDX-1 and GLUT2 gene expression more than 2-fold. Interestingly, GB pretreatment per se did not change the expression of the 2 genes. Addition of SVS to GB, increased PDX-1 but not GLUT2 expression. On the other hand, GB inhibited the SVS-induced increase in the transcription of PDX-1 and GLUT2.

Recently, Ahlgren and coworkers [33] demonstrated a striking reduction in GLUT2 expression in the islets of PDX-1 heterozygote mice and suggested that impaired expression of GLUT2 might be a general cause of hyperglycemia and T2DM. The expression of PDX-1 and GLUT2 in islets from PDX-$1^{+/-}$ mice was reduced to 68% and 55%, respectively, and the in situ perifused pancreas of mice secreted about 45% less insulin to a glucose stimulation (16.7 mmol/L glucose) [11]. These data corroborate well with our observations that SVS pretreatment increased both PDX-1 and GLUT2 gene expressions and that this was accompanied by enhanced GSIS and insulin content. Tiedge and Lenzen [13] found that 1-hour GB treatment did not normalize GLUT2 messenger RNA reduction induced by 2 days' fasting. We also observed that GB alone did not change PDX-1 nor GLUT2 gene expressions, implying that these 2 genes may not play an important role in the GB desensitization. We found that GB in combination with SVS decreased the SVS-induced increase in PDX-1 and GLUT2 expression, indicating a deleterious effect of GB.

In conclusion, SVS does not influence BIS but counteracts the suppressed glucose-stimulated insulin release induced by GB. Both increased insulin content and effects of SVS on PDX-1 and GLUT2 gene expressions may play a role in this beneficial phenomenon. SVS may be able to ameliorate the GB-induced secondary failure. It should be stressed that the desensitization found in vitro needs to be confirmed in long-term and in vivo studies to verify that the positive effects of SVS are preserved and play an important clinical role.

Acknowledgments

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References

[1] Anonymous. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. Lancet 1998;352:854-65.

[2] Anonymous. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet 1998;352:837-53.

[3] Groop LC. Sulfonylureas in NIDDM. Diabetes Care 1992;15:737-54.

[4] DeFronzo RA, Simonson DC. Oral sulfonylurea agents suppress hepatic glucose production in non–insulin-dependent diabetic individuals. Diabetes Care 1984;7(Suppl 1):72-80.

[5] Duckworth WC, Solomon SS, Kitabchi AE. Effect of chronic sulfonylurea therapy on plasma insulin and proinsulin levels. J Clin Endocrinol Metab 1972;35:585-91.

[6] Fineberg SE, Schneider SH. Glipizide versus tolbutamide, an open trial. Effects on insulin secretory patterns and glucose concentrations. Diabetologia 1980;18:49-54.

[7] Wajchenberg BL, Nery M, Leme CE, et al. Effect of prolonged glyclazide treatment on blood glucose and plasma insulin responses in obese patients with maturity-onset diabetes. Clin Pharmacol Ther 1980;27:375-8.

[8] Matthews DR, Cull CA, Stratton IM, et al. UKPDS 26: sulphonylurea failure in non–insulin-dependent diabetic patients over six years. UK Prospective Diabetes Study (UKPDS) Group. Diabet Med 1998;15:297-303.

[9] Anello M, Gilon P, Henquin JC. Alterations of insulin secretion from mouse islets treated with sulphonylureas: perturbations of Ca^{2+} regulation prevail over changes in insulin content. Br J Pharmacol 1999;127:1883-91.

[10] Ball AJ, Flatt PR, McClenaghan NH. Desensitization of sulphonylurea- and nutrient-induced insulin secretion following prolonged treatment with glibenclamide. Eur J Pharmacol 2000;408:327-33.

[11] Brissova M, Shiota M, Nicholson WE, et al. Reduction in pancreatic transcription factor PDX-1 impairs glucose-stimulated insulin secretion. J Biol Chem 2002;277:11225-32.

[12] Clocquet AR, Egan JM, Stoffers DA, et al. Impaired insulin secretion and increased insulin sensitivity in familial maturity-onset diabetes of the young 4 (insulin promoter factor 1 gene). Diabetes 2000;49:1856-64.

[13] Tiedge M, Lenzen S. Effects of glucose refeeding and glibenclamide treatment on glucokinase and GLUT2 gene expression in pancreatic B-cells and liver from rats 1. Biochem J 1995;308(Pt 1):139-44.

[14] Chan P, Tomlinson B, Chen YJ, et al. A double-blind placebo-controlled study of the effectiveness and tolerability of oral stevioside in human hypertension. Br J Clin Pharmacol 2000;50:215-20.

[15] Chan P, Xu DY, Liu JC, et al. The effect of stevioside on blood pressure and plasma catecholamines in spontaneously hypertensive rats. Life Sci 1998;63:1679-84.

[16] Gregersen S, Jeppesen PB, Holst JJ, et al. Antihyperglycemic effects of stevioside in type 2 diabetic subjects. Metabolism 2004;53:73-6.

[17] Hong J, Chen L, Jeppesen PB, et al. Stevioside counteracts the {alpha}-cell hypersecretion caused by long-term palmitate exposure. Am J Physiol Endocrinol Metab 2006;290:E416-22.

[18] Jeppesen PB, Gregersen S, Rolfsen SE, et al. Antihyperglycemic and blood pressure-reducing effects of stevioside in the diabetic Goto-Kakizaki rat. Metabolism 2003;52:372-8.

[19] Jeppesen PB, Gregersen S, Poulsen CR, et al. Stevioside acts directly on pancreatic beta cells to secrete insulin: actions independent of cyclic adenosine monophosphate and adenosine triphosphate-sensitive K^+-channel activity. Metabolism 2000;49:208-14.

[20] Chen J, Jeppesen PB, Abudula R, et al. Stevioside does not cause increased basal insulin secretion or beta-cell desensitization as does the sulphonylurea, glibenclamide: studies in vitro. Life Sci 2006;78:1748-53.

[21] Lacy PE, Kostianovsky M. Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes 1967;16:35-9.

[22] Gregersen S, Hermansen K, Yanaihara N, et al. Galanin fragments and analogues: effects on glucose-stimulated insulin secretion from isolated rat islets 1. Pancreas 1991;6:216-20.

[23] Jeppesen PB, Gregersen S, Alstrup KK, et al. Stevioside induces antihyperglycaemic, insulinotropic and glucagonostatic effects in vivo: studies in the diabetic Goto-Kakizaki (GK) rats. Phytomedicine 2002;9:9-14.
[24] Filipponi P, Marcelli M, Nicoletti I, et al. Suppressive effect of long term sulfonylurea treatment on A, B, and D cells of normal rat pancreas. Endocrinology 1983;113:1972-9.
[25] Henquin JC. Tolbutamide stimulation and inhibition of insulin release: studies of the underlying ionic mechanisms in isolated rat islets. Diabetologia 1980;18:151-60.
[26] Rabuazzo AM, Buscema M, Vinci C, et al. Glyburide and tolbutamide induce desensitization of insulin release in rat pancreatic islets by different mechanisms. Endocrinology 1992;131:1815-20.
[27] Ikegami H, Shima K, Tanaka A, et al. Interindividual variation in the absorption of glibenclamide in man. Acta Endocrinol (Copenh) 1986;111:528-32.
[28] Kawashima K, Kuzuya T, Matsuda A. Radioimmunoassay of glibenclamide. Diabetes 1979;28:221-6.
[29] Del PS, Marchetti P. Beta- and alpha-cell dysfunction in type 2 diabetes. Horm Metab Res 2004;36:775-81.
[30] Rustenbeck I. Desensitization of insulin secretion. Biochem Pharmacol 2002;63:1921-35.
[31] Bratanova-Tochkova TK, Cheng H, Daniel S, et al. Triggering and augmentation mechanisms, granule pools, and biphasic insulin secretion. Diabetes 2002;51(Suppl 1):S83-S90.
[32] Kemp DM, Habener JF. Synergistic effect of dimethyl sulfoxide on glucagon-like peptide 1 (GLP-1)-stimulated insulin secretion and gene transcription in INS-1 cells: characterization and implications. Biochem Pharmacol 2002;64:689-97.
[33] Ahlgren U, Jonsson J, Jonsson L, et al. Beta-cell–specific inactivation of the mouse Ipf1/Pdx1 gene results in loss of the beta-cell phenotype and maturity onset diabetes. Genes Dev 1998;12:1763-8.