Polysaccharide isolated from *Triticum aestivum* stimulates insulin release from pancreatic cells via the ATP-sensitive K+ channel

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Received November 30, 2011; Accepted January 18, 2012

DOI: 10.3892/ijmm.2012.905

**Abstract.** Traditional natural plants have been used throughout the world for their antidiabetic effects. The aim of the present study was to investigate the stimulating activity of a polysaccharide extract derived from *T. aestivum* sprout (TASP) on insulin secretion in vitro using the RIN-5F pancreatic β-cell line and rat pancreatic islets. In these experiments, TASP (0.1 to 2 mg/ml) augmented glucose-stimulated insulin secretion in a dose-dependent manner in the presence of a stimulatory glucose concentration (16.7 mM), but not of a basal concentration (1.1 mM). Although TASP failed to enhance the high K+-induced insulin secretion, the insulinotropic effect of TASP was significantly inhibited by diazoxide, an opener of ATP-sensitive K+ channel blocking insulin release. TASP potentiated the insulin secretion induced by other secretagogues, such as IBMX and tolbutamide. Moreover, glucose-derived blood insulin levels were significantly elevated by oral administration of TASP to mice, similarly to antidiabetic drugs. We also demonstrated that TASP significantly increased glucose-induced 45Ca2+ uptake and proinsulin mRNA expression in rat islets. Overall, our results suggest that TASP has a stimulating effect on insulin secretion and production in pancreatic β-cells via K+ channel closure and calcium influx. These results suggest that TASP may be useful as a candidate for the therapy of diabetes mellitus.

**Introduction**

Diabetes mellitus is a metabolic disease characterized by high blood glucose levels. Diabetic patients are classified into 2 types; type 1 diabetes results from a deficiency in insulin secretion, whereas type 2 diabetes is a combination of insulin resistance and inadequate insulin secretion. Insulin is a hormone produced by β-cells in the pancreas after exposure to hyperglycemic conditions, and then acts to trigger tissues in the body to absorb glucose from the blood. A lack of insulin in the case of diabetes leads to severe dysfunction in the target organs as muscle, liver and adipose tissue (1,2). The mechanisms of glucose-dependent insulin secretion have been examined by applying β-cells blockers (3). Uptake of glucose into β-cells leads to an elevation of ATP cellular level and blockage of the K+ channels that results at the membrane depolarization, Ca2+ influx, and subsequent insulin secretion by exocytosis (4,5).

In the pharmacotherapy of diabetes mellitus and insulin-resistance, insulin is administered as the main treatment to reduce excess blood glucose levels. Also, sulphonylureas can be used to trigger insulin release from β-cells through opening of calcium channels (6). However, sulphonylureas have been prescribed with many restrictions due to side-effects, leading to hypoglycemia via the gradual β-cell destruction and impaired endothelial cell function (7). Most of the current drugs used for type 2 diabetes patients are not free from side effects and do not restore normal glucose homeostasis (8). Thus, the strategies of antidiabetic drug development focus on controlling glucose homeostasis with low toxicity even after extended use. Recent studies have been focused on herbal plants which are used traditionally as the source of foods, medicines, or nutritional supplements, for maintaining and treating diabetes.

A variety of polysaccharides from different biological sources has been postulated to enhance insulin release and sensitivity in diabetes. Since numerous bioactive polysaccharides isolated from herbal plants have been shown to have antidiabetic effects, they are becoming attractive materials for additional pharmaceutical products. Generally, the dietary supplements containing high levels of water soluble polysaccharides, such as soluble fiber and β-glucan, are effective in improving serum lipid levels, blood glucose levels, and insulin resistance (9-11). Current studies have shown that polysaccharides isolated from the traditional medical herbs have antihyperglycemic effect by insulin secreting activity.
(12,13). We previously have shown that the aqueous extracts of *Triticum aestivum* sprout (TASP) attenuated blood glucose level in the streptozotocin (STZ)-induced diabetic mice, which is an animal model of human insulin-dependent type 1 diabetes (14). Some studies have shown that the aqueous extracts from wheat bran and wheatgrass improved postprandial glucose metabolism in patients with type 2 diabetes and diabetic rats (15-17). However, the hypoglycemic mechanisms of the aqueous extracts of TASP in diabetic mice remain completely unclear. Wheat is one of the most produced corps in the world, along with corn and rice. During the germination/sprouting stage, the synthesis of useful compounds, such as vitamins and phenolic compounds, occurs in the seeds. The germinated wheat leaves generally are called wheatgrass, in some European countries, USA and India, and consumed as a health food due to a rich source of soluble fibers, vitamins, antioxidants and minerals (18).

In the present study, according to evidence that plant-derived polysaccharides have stimulatory effects on the insulin response (19,20), we investigated the stimulatory effects of water soluble polysaccharide derived from TASP on insulin release from the pancreatic cells *in vitro* and *in vivo*. We also elucidated the involvement of the ATP-sensitive K⁺ channel and Ca²⁺ influx with TASP using the pancreatic cells and rat islets, which have been used for the study of insulin secretogogues.

### Materials and methods

**Cell culture.** The RIN-5F cell line was purchased from the ATCC Global Bioresource Center (Manassas, VA). Cells were maintained in RPMI-1640 medium (HyClone, Logan, UT) containing 10% fetal bovine serum (HyClone) and 100 U/ml penicillin/streptomycin (Invitrogen, Carlsbad, CA) by transfer to new culture plates every 2 days. Male Wistar rats (9-10 weeks, 240-260 g body weight) were euthanized by inhalation of ether and cervical dislocation. The pancreas was dissected out and the islets of Langerhans were isolated by the collagenase digestion method (21). Isolated islets were collected in new tube and washed twice with Kreb’s-Ringer bicarbonate (KRB) buffer (115 mM NaCl, 4.7 mM KCl, 1.28 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 24 mM NaHCO₃, 1.1 mM glucose, 0.25% bovine serum albumin; pH 7.4), and preincubated for 1 h at 37°C under 5% CO₂ atmosphere. The islets, 914 beta cells/well, were seeded in 96-well plates and then incubated in Kreb’s solution containing 1.1 or 16.7 mM glucose with or without compounds, and then 10 µl of the CCK-8 solution was added into each well for further incubated for 2 h. The absorbance at 450 nm was measured using a microplate reader. Data are shown as the percentage of cell viability.

**Insulin assay.** The RIN-5F pancreatic cell line was used to evaluate the activity of insulin secretion. Cells (2.0x10⁴ cells/well) were seeded in the plate. After 24 h, each well was washed twice with KRB buffer and then replaced with fresh KRB buffer containing 1.1 or 16.7 mM glucose with or without various reagents or the extract. After incubation for 60 min, the medium was collected and centrifuged to remove the cells. The supernatant was used for insulin measurement. We also elucidated the involvement of the ATP-sensitive K⁺ channel and Ca²⁺ influx with TASP using the pancreatic cells and rat islets, which have been used for the study of insulin secretogogues.

**Extraction and purification of TASP.** TASP were provided from the National Institute of Crop Science (Jeonbuk, Korea) and acclimated for 4 weeks prior to use. All mice were housed under standard conditions at 22±1°C with humidity of 50±10%. All procedures were carried out in strict according to the guidelines established by the University's Committee for Cytotoxicity assay. Cell cytotoxicity assays were performed using the cell counting kit-8 (CCK-8) (Djodji Molecular Technology, Inc., Rockville, MD). Cells (2.0x10⁴ cells/well) were seeded in 96-well plates and incubated with conditioned RPMI-1640 medium at 37°C under 5% CO₂ atmosphere. After preincubation for 2 h, the RIN-5F cells were cultured with or without compounds, and then 10 µl of the CCK-8 solution was added into each well for further incubated for 2 h. The absorbance at 450 nm was measured using a microplate reader. Data are shown as the percentage of cell viability.

**4Ca²⁺ uptake assay.** To examine the involvement of calcium influx, the Ca²⁺ uptake assay was performed in rat islets as described previously (25). Briefly, the isolated 10 rat islets were seeded in 96-well plates and then incubated in Kreb’s solution containing 1.1 or 16.7 mM glucose with or without various reagents or the extract. After incubation for 60 min, the medium was collected and centrifuged to remove the cells. The supernatant was used for insulin measurement and the insulin content was determined by an enzyme linked immunosorbent assay (ELISA) kit obtained from Shibayagi Co. (Ishihara, Japan).

**In vivo experiments.** Male C57BL/6 mice (8-weeks-old) were purchased from Chungang Experimental Animal, Inc. (Seoul, Korea) and acclimated for 4 weeks prior to use. All mice were housed under standard conditions at 22±1°C with humidity of 50±10%. All procedures were carried out in strict according to the guidelines established by the University's Committee for...
Animal Experiments regarding the use and care of laboratory animals. Mice were fasted for 18 h before the experiments. For the glucose tolerance test, glucose (2 g/kg) was given orally to fasted mice with or without TASP, which was administered orally 30 min before glucose challenge. Blood samples were obtained from the tail vein of mice before and 30, 60, 90, 120, 150 and 180 min after glucose challenge. Blood glucose levels were determined at all time points using the Accu-Chek blood glucose meter (Roche Diagnostics GmbH, Mannheim, Germany). The serum insulin levels were measured in blood samples obtained from the orbital sinus 60 min after glucose challenge by an ELISA kit.

**RNA isolation and real-time RT-PCR.** Total-RNA was extracted from rat islets by TRIzol reagent (Invitrogen), precipitated with isopropanol. RNA (1 μg) was used for synthesizing list-strand cDNA using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). The specific primers of insulin and β-actin used for PCR were designed through the Primer Express Software (Applied Biosystems, Foster City, CA); insulin, forward primer, 5'-TCT TCA GAC CTT GGC ACT GGA-3' and reverse primer, 5'-AGA TGC TGG TGC AGC ACT GAT-3'; β-actin, forward primer, 5'-ACG AGG CCC AGA GCA AGA-3' and reverse primer, 5'-TTG GTT ACA ATG CCG TGT TCA-3'; β-actin was used as a control gene. The PCR reactions were performed on 384-well plates using the ABI 7900HT Fast Real-Time PCR System (Applied Biosystems).

**Statistical analysis.** All values are represented as the mean ± SEM. The statistical significance of the experiments was evaluated by the one-way ANOVA test. P<0.05 was considered to be significant.

**Results**

**The effect of TASP on insulin secretion in RIN-5F cells.** We investigated the stimulatory effect of water soluble polysaccharide-rich TASP on insulin secretion in vitro to estimate its antidiabetic potential. The activity was estimated based on the insulin levels released from RIN-5F cells under both basal and hyperglycemic conditions, which contain 1.1 and 16.7 mM glucose, respectively. As shown in Fig. 1, the insulin secretion was augmented when the glucose concentration was increased from 1.1 to 16.7 mM. Although TASP in the presence of 1.1 mM glucose did not induce the insulin secretion from the cells, exposure to TASP (0.1-2 mg/ml) in the presence of 16.7 mM glucose induced a significant increase in insulin secretion in a concentration-dependent manner (P<0.01). The maximum activity was observed with 1 mg/ml TASP. Indeed, at 16.7 mM glucose, TASP induced a 2.7-fold increase in the insulin secretion compared to that without TASP; whereas TASP in 1.1 mM glucose showed a lower activity. Toxicity profiles of TASP in the cells were determined at the 0.01-10 mg/ml range of TASP concentration by a cell proliferation assay (Fig. 2). The incubation period for the induction of insulin secretion was insufficient to detect cytotoxicity. Therefore, the cultures were incubated for 24 h. TASP did not show apparent cytotoxicity up to 5 mg/ml, indicating that the activities of TASP on insulin secretion were not due to their toxicity.

**The effect of TASP on insulin secretion in the presence of K⁺.** We examined the influence of membrane depolarization using a high concentration of K⁺ on the insulin secretion induced by TASP (Fig. 3). As shown in Fig. 3A, a depolarizing concentration of KCl (30 mM) significantly augmented the insulin secretion at both low and high glucose concentrations in the absence of TASP (P<0.01). Indeed, exposure to high K⁺ in the presence of 1.1 and 16.7 mM glucose induced a 3.3- and 2.6-fold increase in insulin release, respectively, whereas the combination of TASP (1 mg/ml) with K⁺ did not show a significant increase in KCl-induced insulin secretion. Next, to examine the involvement of K⁺ channel closure in cell membranes on the increase in insulin secretion by TASP, we used diazoxide (300 μM), which is a well known opener of the K⁺-ATP channel (26). As shown in Fig. 3B, treatment with diazoxide induced a prominent inhibition of insulin release at all conditions. Indeed, incubation with diazoxide in the presence of 16.7 mM glucose induced a significant decrease (61%) in TASP-induced insulin secretion.
release (P<0.01). In addition, the inhibitory effect of diazoxide indicates that the stimulation of insulin secretion by TASP was not a consequence of damage to the cells.

Effect of TASP in the presence of insulin secretagogues. We examined the interaction of TASP with insulin secretagogues, such as L-alanine, 3-isobutyl-1-methylxantine (IBMX, a phosphodiesterase inhibitor), and tolbutamide (an inhibitor of β-cell K⁺-ATP channels), on glucose-induced insulin release (7,27,28). As shown in Fig. 4, exposure to L-alanine (10 mM), IBMX (25 mM) or tolbutamide (0.2 mM) induced 2.1-, 1.3- or 2.0-fold increases in insulin release in the presence of 1.1 mM glucose (Fig. 4A), and 2.7-, 1.3- or 3.7-fold increases in the presence of 16.7 mM glucose (Fig. 4B), respectively. TASP (1 mg/ml) significantly enhanced their activities on insulin secretion (P<0.05, P<0.01). The synergistic effect of TASP on the insulin secretion was observed by combination with L-alanine, IBMX or tolbutamide in the presence of 1.1 mM glucose (Fig. 4A). However, although L-alanine and IBMX stimulated the increase in insulin secretion from the cells, their combination with TASP showed no synergistic effect in the presence of 16.7 mM glucose (Fig. 4B). Tolbutamide also revealed an insignificant increase in TASP-stimulated insulin secretion in the presence of 16.7 mM glucose, compared to that with TASP alone. The results suggest that the stimulatory effect of TASP may be similar to its mechanism of inducing insulin secretion.

Effect of TASP on Ca²⁺-uptake and proinsulin mRNA expression. Some insulin secretagogues increase insulin secretion and expression by allowing the extracellular Ca²⁺ flux into β-cells (25). We therefore examined whether TASP influences Ca²⁺ uptake and insulin expression in the pancreatic islets isolated from rats. To assess the activity of Ca²⁺ uptake, the isolated rat islets were stimulated with or without TASP (1 mg/ml) in

### Table 1. Effect of TASP on Ca²⁺ uptake into the isolated rat pancreatic islets in the presence of 1.1 and 16.7 mM of glucose.

<table>
<thead>
<tr>
<th>Glucose (mM)</th>
<th>Control</th>
<th>TASP</th>
</tr>
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<tbody>
<tr>
<td>1.1</td>
<td>12,732±1,772</td>
<td>18,899±1,558*</td>
</tr>
<tr>
<td>16.7</td>
<td>21,686±2,726</td>
<td>43,351±2,874*</td>
</tr>
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The isolated rat islets was incubated with or without TASP (1 mg/ml) for 30 min in the presence of 1.1 and 16.7 mM of glucose. Values are presented as the means ± SEM of 5 replicate experiments in each group. *P<0.05, **P<0.01 compared without TASP at the same glucose condition.
the presence of 1.1 and 16.7 mM glucose. As shown in Table I, 

calcium uptake was augmented by 1.7-fold when the glucose

concentration was increased from 1.1 to 16.7 mM. Stimulation

with TASP significantly potentiated the level of glucose-
dependent calcium influx (P<0.01). Indeed, the increase of calcium

influx induced by TASP was 1.48- and 2.0-fold in the presence

of 1.1 and 16.7 mM glucose, respectively. Next, to measure

the level of proinsulin mRNA using real-time PCR, the islets

were stimulated with or without TASP (0, 0.25, 1 mg/ml) for

18 h in the presence of 1.1 and 16.7 mM glucose. As shown

in Fig. 5, the high glucose condition (16.7 mM) resulted in a

2.3-fold increase in the level of proinsulin mRNA compared to

the basal glucose condition (1.1 mM). Moreover, TASP sig-

significantly augmented proinsulin mRNA expression in the islets in a

concentration-dependent manner (P<0.01). Indeed, the maximal

level of proinsulin mRNA was observed in the islets stimulated

with 1 mg/ml of TASP, revealing a 4.4-fold increase in the pres-

ence of 16.7 mM glucose.

Effect of TASP on the levels of blood glucose and serum insulin in vivo. We examined the effects of TASP on blood

glucose regulation and insulin secretion in vivo by the glucose
tolerance test. TASP (25 and 100 mg/kg) was administered

orally 30 min before glucose challenge in fasted normal mice.

As shown in Fig. 6A, the blood glucose level reached a peak

30 min after glucose challenge and decreased in a time-
dependent manner. TASP showed a hypoglycemic effect in

glucose-challenged mice. Indeed, administration of 100 mg/kg

TASP significantly reduced the blood glucose levels elevated

after glucose challenge (P<0.05). Moreover, TASP (100 mg/kg)

induced a significant increase in the levels of blood insulin in

glucose-loaded mice compared to a negative control (P<0.05).

The stimulatory activity of TASP was comparable to that of
tolbutamide, which was used as a positive control. These results

indicate that TASP may have an antihyperglycemic effect by

potentiating glucose-induced insulin release.

Discussion

Traditional plant treatments have been used throughout the

world for the therapy of diabetes mellitus. We previously

demonstrated that the aqueous extract of TASP has a hypogly-
cemic effect in diabetic mice through the increase in serum

insulin concentration (14). In this study, we demonstrated that

TASP potentially has a stimulatory effect on glucose-induced

insulin secretion in vitro and in vivo and in a dose-dependent

manner. The antidiabetic effects of TASP are similar to those

of well known insulin secretagogues, such as tolbutamide. In

addition, TASP significantly enhanced the expression of

proinsulin mRNA in a glucose-dependent response.

Most insulin secretagogues stimulate insulin secretion

from the pancreatic ß-cells through depolarization of the plasma

membrane and activation of calcium-dependent processes

(4). This is established from the mechanism of action of drugs

that are used for therapy of type 2 diabetes patients. It has been

well known that tolbutamide stimulates insulin secretion from

islets, principally by inhibiting ATP-sensitive potassium channels in

the cell membrane, consequently causing a membrane depolar-

ization and an activation of voltage-gated calcium influx (26,29).

It has been known that diazoxide is a compound preventing

the closure of voltage potassium ATP channels and blocking insulin

secretions (30).

In the present study, we observed that diazoxide remarkably

suppressed the stimulating activity of TASP in glucose-induced

insulin release. The results suggest that the ability of TASP to
stimulate insulin secretion may be mediated by depolarization of the cell membrane and consequent closure of K⁺-ATP channels. Interestingly, TASP significantly increased the insulin secretion induced by other insulin secretagogues, such as the inhibitor of cAMP phosphodiesterase (IBMX) and antidiabetic drugs in the presence of basal glucose concentration. However, they failed to potentiate the insulin-releasing effect of TASP in the hyperglycemic condition, indicating the possibility that TASP may have various actions to induce insulin secretion from β-cells.

The calcium entry plays a key role in the insulinotropic activity of pancreatic β-cells (25,31). Thus, we estimated the involvement of calcium by TASP in conditions designed for Ca²⁺ influx into β-cells. Indeed, TASP induced the significant increase in the level of Ca²⁺ uptake into the isolated rat islets in the presence of stimulatory glucose concentrations, revealing that the insulinotropic action induced by TASP is dependent on extracellular calcium flux. The additional involvement of calcium entry was evidenced by the inhibition of the insulin releasing effect caused by the removal of calcium. Interestingly, TASP augmented the expression of proinsulin mRNA in pancreatic β-cells. Although tolbutamide belonging to the sulfonylureas group has been used successfully as an antidiabetic drug for type 2 diabetes due to its property to accelerate the insulin secretion in the pancreas, it has never been used in the treatment of type 1 diabetes due to its ineffective production of insulin (7). In contrast, TASP has the effective properties of stimulating insulin production as well as insulin secretion, suggesting that TASP may be useful as a potent antidiabetic material for the therapy of both type 1 and 2 diabetes.

The insulin secretion effects have been reported in a number of studies using aqueous extracts of plants. Most of the plants with antidiabetic properties have been found to contain compounds such as glycosides, alkaloids, and flavonoids, showing potentially antihyperglycemic action (32-36), but most of them still need proper scientific evaluation. Indeed, the alkaloids and saponins have been reported to inhibit glucose uptake into the isolated rat islets in vitro, whereas the flavonoids could protect various cell types from oxidative stress-mediated cell injury (37,38). Another research has been reported that glycosides could stimulate insulin secretion as well. The polysaccharide compounds derived from algae extracts and Agaricus blazei enhanced the insulin secretion in vitro in the pancreatic β-cells (19,20). Oat bran-derived non-starch polysaccharides acted as an active ingredient decreasing the postprandial hyperglycemic response in humans with type 2 diabetes (39). The levan polysaccharide also was efficient in inhibiting hyperglycemia and oxidative stress induced by diabetes (40). A similar result was observed in humans after eating β-glucan-enriched breads (41). The polysaccharides obtained from the tuberous root of Liriope spicata had the hypoglycemic effects in streptozotocin-induced type 2 diabetic mice (42). These substances may be responsible for the antidiabetic effects of TASP observed in this study.

In conclusion, the present study demonstrated that TASP significantly potentiated the insulin secretion and production from the pancreatic β-cells in a glucose-dependent manner. The insulin secreting activity of TASP may involve the closure of K⁺-ATP channels and the enhancement of the calcium influx through voltage-dependent Ca²⁺ channels, like tolbutamide, which is an antidiabetic drug. Thus, our results indicate that TASP should be regarded as a potent antihyperglycemic agent with the property of accelerating the secretion and production of insulin in the pancreas. TASP has been commonly used as a nutritional supplement controlling diabetes mellitus and its complications. Therefore, further studies are necessary to elucidate the precise mechanisms of the antidiabetic action of TASP, such as insulin signaling, β-cell function, and glucose absorption and metabolism.

Acknowledgements

This study was supported by a grant from the Korea Institute of Planning and Evolution for Technology of Food, Agriculture, Forestry and Fisheries (108156-03-SB010).

References


