Antihyperglycemic Effects of Stevioside in Type 2 Diabetic Subjects

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Stevioside is present in the plant *Stevia rebaudiana Bertoni* (SrB). Extracts of SrB have been used for the treatment of diabetes in, for example, Brazil, although a positive effect on glucose metabolism has not been unequivocally demonstrated. We studied the acute effects of stevioside in type 2 diabetic patients. We hypothesize that supplementation with stevioside to a test meal causes a reduction in postprandial blood glucose. Twelve type 2 diabetic patients were included in an acute, paired cross-over study. A standard test meal was supplemented with either 1 g of stevioside or 1 g of maize starch (control). Blood samples were drawn at 30 minutes before and for 240 minutes after ingestion of the test meal. Compared to control, stevioside reduced the incremental area under the glucose response curve by 18% ($P = .013$). The insulinogenic index ($AUC_{i,insulin}/AUC_{i,glucose}$) was increased by approximately 40% by stevioside compared to control ($P < .001$). Stevioside tended to decrease glucagon levels, while it did not significantly alter the area under the insulin, glucagon-like peptide 1, and glucose-dependent insulinotropic polypeptide curves. In conclusion, stevioside reduces postprandial blood glucose levels in type 2 diabetic patients, indicating beneficial effects on the glucose metabolism. Stevioside may be advantageous in the treatment of type 2 diabetes.

EXTRACTS OF THE plant *Stevia rebaudiana Bertoni* (SrB) have been used for many years in the treatment of diabetes in South America.[1] However, little experimental work has been performed either to prove the clinical efficacy of these extracts in type 2 diabetes, to identify the potential active substance(s) in the extracts, or to reveal the mode of action. Curi et al[2] showed that oral intake of extracts of SrB for 3 days slightly suppressed plasma glucose during an oral glucose tolerance test in healthy subjects. A 35% reduction in blood glucose was also observed in diabetic subjects after oral intake of extracts from SrB.[3] Schmeling et al[4] found a positive impact of extracts on glycemic control in animal diabetes. One of the main constituents of the dry-matter of SrB is the diterpene glycoside, stevioside, which is known for its intense sweetness and has been applied as a noncaloric sweetener in several countries, such as Japan. A number of animal studies have been conducted to investigate the effects of stevioside. Recently, we demonstrated that both intravenously[5] and orally administered stevioside[6] exert antihyperglycemic, insulinotropic, and glucagonostatic actions in the mild type 2 diabetic Goto-Kakizaki (GK) rat. Stevioside and its aglucone steviol directly potentiate the glucose-stimulated insulin secretion from isolated mouse islets.[7] Using patch clamp technique, we revealed that adenosine triphosphate (ATP)-sensitive potassium channels are not involved in the signal transduction cascade within the β cells. This finding is interesting because of the potential disadvantage of treating diabetic patients suffering also from ischemic heart disease with sulfonylureas due to the concomitant closure of the ATP-sensitive potassium channels in heart myocytes.[8] Consequently, stevioside may have advantages compared to sulphonylureas in the treatment of diabetes. Of great importance is also the recent observation that stevioside possesses blood pressure–lowering effects in nondiabetic subjects with hypertension.[9] We hypothesize that stevioside taken with a test meal exerts a lowering of the postprandial blood glucose response.

MATERIALS AND METHODS

Design

The protocol was approved by the local ethical committee at Aarhus County. Patients were recruited from the outpatient clinic at Aarhus University Hospital. All patients gave written informed consent. After a general examination the patients were included according to the following inclusion criteria: (1) type 2 diabetes (treated by diet and/or oral antidiabetic medication); (2) diabetes onset at age greater than 40 years; (3) diabetes duration longer than 1 year and hemoglobin A_{1C} (HbA$_{1C}$) less than 10%; and (4) body mass index between 25 and 32 kg/m^2. The exclusion criteria were (1) treatment with insulin within the last 6 months; (2) enrollment in a clinical trial of drugs within the last 3 months; (3) significant cardiovascular, psychological, neurological, renal, or endocrine disease (apart from diabetes), alcohol or drug abuse, or acute illness; (4) fasting levels of glucose less than 4 or greater than 12 mmol/L on the day of the experiment; and (5) treatment with glucocorticoids.

The test meals were served at 8 AM in random order on 2 separate days at least 1 week apart. The patients were instructed to fast for 10 hours and to abstain from smoking. The subjects were sedentary during the experimental period of 4½ hours. The patients treated with oral antidiabetic medication did not take these tablets prior to the experiments. The height and weight of the patients were noted. An intravenous cannula was placed in an antecubital vein and blood samples were drawn at time points -30, 0, 10, 20, 30, 45, 60, 90, 120, 180, and 240 minutes. The following parameters were determined in plasma: glucose, insulin, triglyceride, free fatty acids, total cholesterol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, glucagon-like peptide-1 (GLP-1), gastric inhibitory polypeptide (GIP), glucagon, glycated hemoglobin A_{1C}, Na$^+$, K$^+$, creatinine, and hemoglobin. The urine volume during the experiment was determined as well as the excretion of albumin, glucose, Na$^+$, and K$^+$. During the test meal blood pressure was measured using automatic blood pressure equipment from SpaceLabs (Issaquah, WA).

Meal

The experiment was a short-term, paired, cross-over study in which each subject received each treatment. A standard test meal was served either with 1 g of stevioside or with 1 g of maize starch (placebo) encapsulated in gelatin and administered orally. The ingested dose was empirically set to 1.0 g. The extracts of leaves of the *Stevia rebaudiana* plant were purchased from Steviafarm Industrial S/A, Maringa, Parana, Brazil (lot. no. 9.80.130). The extract contained 91% of pure stevioside, 4% of rebaudioside A, and 5% of other stevioside derivates according to the manufacturer. The plants used for the production originate from Paraguay and Brazil (Amanbay mountain area).

During the experiments and in the days after no hypoglycemic or other adverse effects were reported by the patients or observed by the investigators.

The total energy content of the test meal was 412 kcal (protein 16 E%, fat 30 E%, carbohydrate 54 E%). The standard carbohydrate-rich test meal consisted of wheat toast (75 g), with 10 g of margarine and cheese (30 g), orange juice (2 dL), and 2 cups of coffee. The total energy content was 1,725 kJ; in percent the energy composition was: 15.8 g protein (16%), 13.6 g fat (30%), and 55.1 g carbohydrate (54%). Each meal contained 4.6 g of dietary fiber.

Analysis

Plasma insulin was measured using a human insulin kit from Linco Research (St Charles, MO). Glucagon, GLP-1, and GIP concentrations in plasma were all measured after extraction of plasma with 70% ethanol (vol/vol, final concentration). For the GIP radioimmunoassay,[10] we used the C-terminally directed antiserum R 65, that cross-reacts fully with human GIP but not with the so-called GIP 8000, whose chemical nature and relationship to GIP secretion is uncertain. Human GIP and ^{125}I human GIP (70 MBq/nmol) were used for standards and tracer. The glucagon radioimmunoassay was directed against the C-terminus of the glucagon molecule (antibody code no. 4305) and therefore mainly measures glucagon of pancreatic origin.[11] The plasma concentrations of GLP-1 were measured[12] against standards of synthetic GLP-1 7-36amide using antiserum code no. 89390, which is specific for the amidated C-terminus of GLP-1 and therefore mainly reacts with GLP-1 of intestinal origin. For these 3 assays, sensitivity was below 1 pmol/L, intra-assay coefficient of variation below 6% at 20 pmol/L, and recovery of standard, added to plasma before extraction, about 100% when corrected for losses inherent in the plasma extraction procedure. Plasma glucose was determined using the glucose GOD-PAP method supplied by Boehringer Mannheim (Mannheim, Germany). Triglyceride, free fatty acids, and total cholesterol were determined using colorimetric kits from Boehringer Mannheim. HbA$_{1C}$ was determined using high-performance liquid chromatography (HPLC).

Statistics

Students paired *t* test was used for comparing the effects of stevioside with placebo on the parameters measured. Data are given as mean ± SEM. The incremental areas under the response curves (AUC$_i$) were calculated. The insulinogenic index was calculated as the ratio between the AUC$_{i,insulin}$ and AUC$_{i,glucose}$. Conversion factors are as follows: for glucose, mg/dL = 0.05551 mmol/L and for insulin, μU/mL = 6.0 pmol/L.

RESULTS

Twelve type 2 diabetic patients (4 females/8 males) with a mean age of 65.8±1.6 years, a diabetes duration of 6.0 ± 1.3 years, a mean body mass index of 28.5 ± 1.0 m^2/kg, and a mean HbA$_{1C}$ of 7.4% ± 0.4% were included in the study. Prior to the study 3 of the patients were treated with diet
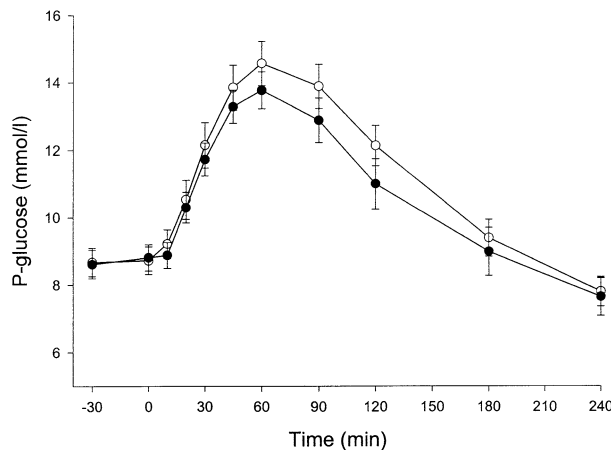

Fig 1. Effects of per orally administered stevioside (compared to control) on postprandial blood glucose excursions in type 2 diabetic patients. One gram of stevioside or maize starch was administered at time point 0 together with a mixed meal. Results are mean ± SEM, n = 12. (●) Stevioside; (○) control.

alone and 9 in addition with tablets (sulfonylurea or metformin alone or in combination). The urine production during the 2 meal tests were 314 ± 42 mL (stevioside) and 352 ± 50 mL (control). The urinary glucose excretion before (<3 mmol/L in all subjects) and during the test meals were not different at the 2 occasions (59 ± 15 mmol/L [stevioside] *v* 60 ± 17 mmol/L [control], *P* = .94). The urinary excretion of sodium and potassium at the 2 occasions were not different.

Stevioside reduced the postprandial blood glucose response by 18% ± 5% (*P* < .004) compared to placebo (AUC$_{i,glucose}$ 638 ± 55 *v* 522 ± 64 mmol/L × 240 min [control *v* stevioside]; *P* < .02) as seen in Fig 1. Stevioside tended to enhance the area under the insulin response curve from time point 0 to 240 minutes (AUC$_{i,insulin}$ 34,991 ± 3,550 *v* 38,499 ± 3,740 pmol/L × 240 min [control *v* stevioside]); however, the difference did not each statistical significance (*P* = .08) (Fig 2). The initial responses from time point 0 to 30 minutes also did not differ (2,118 ± 338 *v* 2,486 ± 395 pmol/L × 30 min [control *v* stevioside]). Stevioside caused a 40% increase in the insulinogenic index compared to control (60 ± 8 [control] *v* 84 ± 11 [stevioside], *P* < .001). Stevioside seemed to reduce the postprandial glucagon levels, while the postprandial GLP-1 and GIP did not significantly differ on the 2 occasions (Table 1). Also, systolic and diastolic blood pressures were not altered by stevioside administration compared to control (Table 1).

Postprandial plasma level of triglycerides increased from time point 0 to 240 minutes with no differences between the 2 treatment groups (Δ values: 0.56 ± 0.09 [control] *v* 0.53 ± 0.11 mmol/L [stevioside]). The level of free fatty acids decreased in both situations, but no difference between groups was found (Δ values 0.15 ± 0.04 [control] *v* 0.13 ± 0.11 mmol/L [stevioside]).
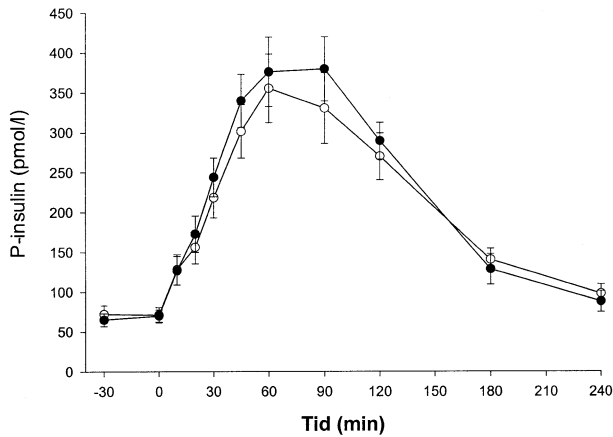
Fig 2. Effects of per orally administered stevioside (compared to control) on postprandial plasma insulin levels in type 2 diabetic patients. One gram of stevioside or maize starch was administered at time point 0 together with a mixed meal. Results are mean ± SEM, n = 12. (●) Stevioside; (○) control.

DISCUSSION

Stevioside suppresses the postprandial blood glucose level in type 2 diabetic subjects by in average 18%. The circulating insulin levels tended to be increased by stevioside but did not attain statistical significance. However, when calculating the insulinogenic index, a significant increase of 40% was found. A direct effect on the β cell may be difficult to detect in the periphery due to dilution. Interestingly, the urinary glucose output was unaffected by the intake of stevioside. Previous studies in rats, however, have indicated that stevioside may increase urinary glucose loss,[13,14] as well as augmenting liver glycogen storage.[15] The reason for the apparent discrepancy is not clear. The present study is unable to clarify if stevioside has direct effects on the peripheral glucose disposal beside that induced by insulin. There are no indications from our study that stevioside should cause late hypoglycemia. In the present study stevioside was administered orally as 91% pure stevioside. Therefore, we cannot totally exclude the possibility that other substances in the extract in part could be responsible for the observed effects.

Studies using the mild type 2 diabetic GK rat have also shown a blood glucose–lowering effect of stevioside both when administered intravenously[5] and when given orally.[6] In one study in normal rats, acute infusion of stevioside was found to increase blood glucose while no negative long-term effect on blood glucose was observed.[16] Our in vitro studies in isolated mouse islets showed glucose-dependent insulin release to stevioside.[7] However, the insulinotropic effect of stevioside in animal experiments faded in the presence of normal to low glucose.[7,17] Also Chan et al[8] found lack of effect on fasting blood glucose of stevioside in hypertensive, nondiabetic subjects. Thus it can be hypothesized that an elevated glucose level, as found in the diabetic state, is needed for stevioside to elicit its beneficial effects. Previously we have shown a glucagonostatic effect of intravenous stevioside in GK rats.[5] A glucagonostatic effect may be beneficial for the glucose metabolism in type 2 diabetes.[18,19] Apparently, stevioside tended to elicit a small reduction in the incremental AUCs of the glucagon and GLP-1 curves. However, the curves disclose considerable, insignificant differences in basal levels of both glucagon and GLP-1 between the 2 test situations, indicating that the differences may not be of functional importance. Thus, in human type 2 diabetic patients we have no clear-cut proof of a glucagonostatic effect of orally administered stevioside. Also, the GIP response curves appeared quite similar. This indicates that stevioside does not seem to act via the entero-insular axis, but via a direct action on β cells action, corroborating our in vitro studies.[7]

Triglyceride and free fatty acid levels were not changed by stevioside, indicating that stevioside does not affect the intestinal nutrient uptake. Corroborating this, stevioside has been found not to alter the uptake of oleic acid from the rat intestine.[20]

There is evidence that stevioside may have beneficial actions on other constituents of the metabolic syndrome than the hyperglycemia. Thus, Chan et al found a blood pressure–lowering effect after long-term oral administration of stevioside to hypertensive, nondiabetic patients.[8] Studies on dose-dependency, pharmacokinetics, and toxicology are needed in humans. Stevioside seems to be a safe compound that has been used for decades as a sweetening agent in several countries such as Japan without adverse effects. Whether long-term administration of stevioside would improve the postprandial glycemia and

Table 1. Effects of Orally Administered Stevioside (compared to control) on Postprandial Levels of Glucagon, GLP-1 GIP (presented as area under the response curves), and Blood Pressure in Type 2 Diabetic Subjects

	Control	Stevioside	P Value
Glucagon (AUC$_i$, pmol/L · 240 min)	348 ± 46	281 ± 33	<.021
GLP-1 (AUC$_i$, pmol/L · 240 min)	2,208 ± 253	1,529 ± 296	NS
GIP (AUC$_i$, pmol/L · 240 min)	7,412 ± 635	6,747 ± 1,023	NS
Mean systolic BP (mm Hg)			
−30 to 0 min	146 ± 4	143 ± 5	NS
15 to 240 min	143 ± 4	138 ± 4	NS
Mean diastolic BP (mm Hg)			
−30 to 0 min	86 ± 3	88 ± 3	NS
15 to 240 min	87 ± 2	84 ± 3	NS

NOTE. One gram of stevioside or maize starch was administered at time point 0 together with a mixed meal. Results are mean ± SEM, n = 12. Abbreviation: NS, not significant.

blood pressure enough to justify its use as a new treatment for type 2 diabetes remains to be proven.

In conclusion, stevioside reduces postprandial blood glucose and tends to potentiate the insulin secretion in type 2 diabetic patients. Our study therefore seems to confirm the anticipations and knowledge of the native people in Paraguay and Brazil. Stevioside is a potential drug or food additive for improving diabetes regulation.

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