Available online at www.sciencedirect.com
SCIENCE DIRECT®
Life Sciences 78 (2006) 1748 – 1753
Life Sciences
www.elsevier.com/locate/lifescie

Stevioside does not cause increased basal insulin secretion or β-cell desensitization as does the sulphonylurea, glibenclamide: Studies in vitro

Jianguo Chen *, Per Bendix Jeppesen, Reziwanggu Abudula, Stig E.U. Dyrskog, Michele Colombo, Kjeld Hermansen

Department of Endocrinology and Metabolism, Aarhus Sygehus THG, Aarhus University Hospital, Tage-Hansens Gade 2, DK-8000 Aarhus C, Denmark

Received 12 April 2005; accepted 10 August 2005

Abstract

We have shown that stevioside (SVS) enhances insulin secretion and thus may have a potential role as antihyperglycemic agent in the treatment of type 2 diabetes mellitus. However, whether SVS stimulates basal insulin secretion (BIS) and/or cause desensitization of beta cells like sulphonylureas (SU), e.g. glibenclamide (GB), is not known. To explore and compare the effects of SVS pretreatment with those of GB and glucagon-like peptide-1 (GLP-1), we exposed isolated mouse islets to low or high glucose for 1 h after short-term (2 h) or long-term (24 h) pretreatment with SVS, GB or GLP-1, respectively. BIS at 3.3 or 5.5 mM glucose were not changed after short-term pretreatment with SVS (10^{-7} M), while it increased about three folds after pretreatment with GB (10^{-7} M). Glucose stimulated insulin secretion (GSIS) (16.7 mM) increased dose-dependently after long-term pretreatment with SVS at concentrations from 10^{-7} to 10^{-5} M. Pretreatment for 24 h with GB (10^{-7} M) increased the subsequent BIS (3.3 mM glucose) ($p<0.001$), but decreased GSIS (16.7 mM glucose) ($p<0.001$). In contrast SVS (10^{-7} M) and GLP-1 (10^{-7} M) did not stimulate BIS but both enhanced the subsequent GSIS (16.7 mM glucose) ($p<0.05$ and $p<0.05$, respectively). While SVS pretreatment increased the intracellular insulin content, GB pretreatment decreased the insulin content. Our study suggests that SVS pretreatment does not cause a stimulation of BIS and does not desensitize β-cells, i.e. SVS seems to have advantageous characteristics to GB as a potential treatment of type 2 diabetes.

Keywords: Stevioside; Basal insulin secretion (BIS); Glucose stimulated insulin secretion (GSIS); Desensitization; Glibenclamide; Glucagon-like peptide-1

Introduction

Type 2 diabetes mellitus is a chronic metabolic disorder that results from both defects in insulin action and insulin secretion. Glucose, one of the key physiological stimuli of the β-cell, increases the cytoplasmic free Ca^{2+} concentration and stimulates insulin secretion. However, chronic elevation of blood glucose concentration impairs β-cell function (Leahy et al., 1986; Ling et al., 1996; Brock et al., 2002) and induces β-cell apoptosis (Efanova et al., 1998). The United Kingdom Prospective Diabetes Study (UKPDS) has demonstrated in type 2 diabetes that complications may be significantly delayed or even prevented with strict glycemic control (1998a,b).

At early stages in type 2 diabetes diet and exercise are usually sufficient to normalize glucose homeostasis, but oral antidiabetic drugs and insulin replacement often become necessary with time due to a decrease of the functional pancreatic β-cell mass in the face of insulin resistance.

Sulphonylureas (SU) have presented the backbone of oral therapy in type 2 diabetes for more than 40 years. They block β-cell ATP-sensitive K^+-channels and lead to membrane depolarization and subsequently insulin secretion (Groop, 1992). SU, e.g. glibenclamide (GB), simulates the effect of glucose in eliciting insulin release (Rosak, 2002) and enhances insulin secretion even at basal glucose levels (Patane et al., 2000) thereby being at risk of causing harmful hypoglycemia (Groop, 1992; Vischer, 1997). Hypoglycemia is the limiting factor in achieving increasingly aggressive targets for good glycemic control in patients with type 2 diabetes. Furthermore, it is known that GB may lose its pharmacological action over time, a phenomenon described as desensitization (Ball et al., 2000). Thus, diabetic subjects often develop secondary failure after long-term treatment with SU (Matthews et al., 1998).

* Corresponding author. Tel.: +45 89497720.
E-mail address: jianguo.chen@ki.au.dk (J. Chen).

0024-3205/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2005.08.012

Given the shortcomings of SU therapy, there is a need for insulin secretagogues with an alternative action profile, i.e. with little or no risk of eliciting hypoglycemia due to insulin release during basal glucose levels and retaining the anti-hyperglycemic action at high glucose.

Stevioside (SVS), a diterpene glycoside ($C_{38}H_{60}O_{18}$) extracted from leaves of the plant *Stevia Rebaudiana Bertoni*, represents a class of insulin secretagogues chemically unrelated to SU. We have previously demonstrated that SVS possesses an acute, glucose dependent insulinotropic effect in isolated mouse islets (Jeppesen et al., 2000). At normal glucose, SVS does not function via closure of ATP-sensitive K^+-channels (Jeppesen et al., 2000). It enhances the first-phase insulin response, suppresses glucagon levels (Jeppesen et al., 2003), and lowers the blood glucose as well as the blood pressure in the type 2 diabetic Goto-Kakizaki (GK) rat and spontaneously hypertensive rat (Hsu et al., 2002; Liu et al., 2003; Jeppesen et al., 2003; Chan et al., 1998). SVS also reduces postprandial blood glucose levels in type 2 diabetic patients (Gregersen et al., 2004).

To explore if SVS possesses an action profile different from SU, i.e. does not cause hypoglycemia or β-cell desensitization, we investigated the impact of SVS on the basal insulin secretion (BIS) and the glucose stimulated insulin secretion (GSIS) after short- or long-term pretreatment with SVS. Furthermore, we compared the effects of SVS with those of glucagon-like peptide-1 (GLP-1), which insulinotropic effect is glucose dependent but disappears at low glucose (D'Alessio and Vahl, 2004; Holst and Gromada, 2004; List and Habener, 2004) as well as with the effects of GB.

Materials and methods

Experimental animals

Adult female NMRI mice (Bomholtgaard Breeding and Research Centre, Ry, Denmark) weighing 20–25 g were used. We fed animals with a standard pellet diet (Altromin, Lage, Germany) with tap water ad libitum before experiments. The light/dark cycle was 12 h. The Danish Council on Animal Care has approved the study.

Isolation and culture of islets

Islets were isolated by the collagenase digestion technique (Lacy and Kostianovsky, 1967). In brief, we anesthetized the animals with pentobarbital (50 mg/kg) intraperitoneally and made a midline laparotomy. The pancreases were filled retrogradely with 3 mL ice-cold Hanks Balanced Salt Solution ([HBSS] Sigma Chemical, St. Louis, MO, USA) supplemented with 0.3 mg/mL collagenase P (Boehringer Mannheim GmbH, Mannheim, Germany). HBSS and collagenase P was filter sterilized before use. Subsequently, we removed the pancreases and incubated them for 19 min at 37 °C in a water bath. After rinsed with ice-cold HBSS, we handpicked the islets under a stereomicroscope and incubated them overnight at 37 °C and 5% CO_2/95% normal atmosphere in 10 mL RPMI 1640. The latter contained 11.1 mM glucose supplemented with 10% fetal calf serum, 2.06 mM L-glutamate, 100 IU/mL penicillin G, 100 μg/mL streptomycin and 0.25 μg/mL amphotericin B (all GIBCO BRL, Paisley, UK).

In short-term pretreatment studies, we replaced the culture medium the following day with 10 mL RPMI containing 5.5 mM glucose alone or supplemented with 10^{-7} M GB or 10^{-7} M SVS, respectively. The islets were cultured for additional 2 h. The long-term pretreatment studies were tripartite. In the first series of experiment, the culture medium was replaced with 10 mL RPMI containing 11.1 mM glucose supplemented with SVS at concentrations between 10^{-8} and 10^{-5} M, respectively. In the second series of long-term experiment, we replaced the culture medium with 10 mL RPMI containing 3.3–22 mM glucose with or without 10^{-7} M SVS. In the third series, we replaced the culture medium with 10 mL RPMI containing 11.1 mM glucose supplemented with 10^{-7} M SVS, 10^{-7} M GB, or 10^{-7} M GLP-1, respectively. We subsequently cultured the islets for 24 h. In these experiments, we added GB (Sigma, St. Louis, MO, USA) to the medium from 10^{-3} M prepared stock solution in dimethylsulfoxid (DMSO) (Merck, Frankfurt, Germany). The final concentration of DMSO in all media was lower than 0.01%. We dissolved SVS (Wako Pure Chemical industries, Osaka, Japan) and GLP-1 (Sigma, St Louis, MO, USA) in distilled water before further dilution in the culture medium.

Insulin secretion studies

After culture, we rinsed islets once with modified Krebs-Ringer buffer (KRB) supplemented with 3.3 mM glucose and 0.1% human serum albumin (Sigma, St. Louis, MO, USA). The KRB contained 125 mM NaCl, 5.9 mM KCl, 1.2 mM $MgCl_2$, 1.28 mM $CaCl_2$, and 25 mM HEPES (pH 7.4; all Sigma). After 30 min preincubation at 37 °C, single islets were handpicked and incubated in 100 μL KRB at glucose concentration (3.3, 5.5 or 16.7 mM) chosen for the respective short-term and the series of long-term experiments (see Results). After 60 min of incubation in normal atmosphere at 37 °C, 50 μL of the medium was collected and frozen for analysis of insulin.

Insulin content

To measure the insulin content, after the third series of long-term culture experiments, we transferred 10 islets to 1 mL of glycine-bovine serum albumin (BSA) buffer (glycine 100 mM, 0.25% BSA, pH 8.8) (Roche Molecular Biochemicals, Mannheim, Germany). Islets were treated with sonication twice at 0 °C for 14 s (Branson Sonifier 250, Danbury, CT, USA). After centrifugation for 30 min at 16,000 rpm, the supernatant was collected and frozen at −20 °C for later insulin assay.

Insulin assay

Insulin was analyzed by radioimmunoassay using a guinea pig anti-porcine insulin antibody (Novo Nordisk, Bagsvaerd, Denmark) and mono-^{125}I-(Tyr A14)-labelled human insulin