Journal of Ethnopharmacology 120 (2008) 190–195

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Korean red ginseng stimulates insulin release from isolated rat pancreatic islets

Kyong Kim, Hye Young Kim*

Food Function Research Center, Food Convergence Technology Division, Korea Food Research Institute, 516 Baekhyun-dong, Bundang-gu, Songnam-si, Kyonggi-do 463-746, Republic of Korea

ARTICLE INFO

Article history:
Received 24 October 2007
Received in revised form 4 August 2008
Accepted 5 August 2008
Available online 15 August 2008

Keywords:
Korean red ginseng
Panax ginseng C.A. Meyer (Araliaceae)
Insulin release
Pancreatic islets

ABSTRACT

Ethnopharmacological relevance: Korean red ginseng (KRG), one of heat-processed Korean ginseng (*Panax ginseng* C.A. Meyer), has a long history as herbal remedy for antidiabetic effect.
Aim of the study: The effect and mechanism of KRG on stimulation of insulin release were investigated in isolated rat pancreatic islets.
Material and methods: Pancreatic islets isolated from rats were used to evaluate the insulinotropic action of KRG. The effect of Ca on the insulinotropic action of KRG was investigated.
Results: The aqueous ethanolic extract of KRG (AEE-KRG) (0.1–1.0 mg/ml) significantly evoked a stimulation of insulin release at 3.3 mM glucose compared to the control. Experiments at different glucose concentrations (8.4 and 16.7 mM) showed that AEE-KRG significantly stimulated on its own whereas it did not potentiate insulin secretion induced by glucose. The extracellular Ca^{2+}-free condition, a L-type Ca^{2+} channel blocker and an ATP-sensitive K^+ channel opener significantly inhibited insulin secretion evoked by AEE-KRG.
Conclusion: These findings suggest that KRG displays beneficial effects in the treatment of diabetes at least in part via the stimulation of insulin release in a glucose-independent manner.

1. Introduction

Historical records reveal that in traditional medical systems, a disease similar to type 2 diabetes was treated with ginseng (Ackerknecht, 1982). The root of the plant *Panax ginseng* C.A. Meyer (Korean ginseng) has been used clinically to treat type 2 diabetes (Bensky and Gamble, 1993; Huang, 1999) and has also been used as a tonic, often taken for years without evidence of adverse effects or toxicity (Lee, 1992; Attele et al., 1999).

Of the two kinds of ginseng, Korean white ginseng (KWG) (*Panax ginseng* C.A. Meyer) is air-dried ginseng, and Korean red ginseng (KRG) (Ginseng Radix Rubra) is produced by steaming raw ginseng at 98–100 °C for 2–3 h. Each type of ginseng has its long history of ethnopharmacological evidence for antidiabetic use. KWG has been reported to exert antidiabetic function in type 1 diabetic animals (Kimura et al., 1981; Waki et al., 1982) and type 2 diabetic animals (Chung et al., 2001; Dey et al., 2003). KRG has been reported to exert antidiabetic function in type 2 diabetic animals (Kim et al., 2005; Liu et al., 2005) and type 2 diabetic patients (Vuksan et al., 2008).

Ginseng might mediate its antidiabetic action through a variety of mechanisms including actions on the insulin-secreting pancreatic β-cells and the target tissues that take up glucose (Xie et al., 2005). A key feature of type 2 diabetes is that glucose fails to stimulate an adequate release of insulin from pancreatic β-cells (Kahn, 2001). Eventually, the pancreatic β-cells fail to compensate for the raised glucose to achieve homeostasis, resulting in overt hyperglycemia.

Insulin secretagogues can be divided into two groups: the initiators and the potentiators. The formers are capable of stimulating insulin secretion on their own and include nutrients, such as glucose, and drugs such as sulfonylureas. All these substances act by inhibiting ATP-sensitive K^+ channel (K_{ATP} channel) activity but whereas the nutrients must be metabolized to effect channel closure, the drugs bind directly to the channel and block its activity. Potentiators of insulin secretion include a number of hormones [for example, glucagons and glucagons-like peptide (GLP1)], transmitters (such as acetylcholine) and the amino acid arginine. These agents amplify insulin secretion induced by an initiator but cannot elicit insulin secretion by themselves because they do not close K_{ATP} channels and are only able to exert their effects after an initiator secretagogue has inhibited K_{ATP} channel. Inhibition of insulin release is produced by agents that open

* Corresponding author. Tel.: +82 31 780 9267; fax: +82 31 709 9876.
E-mail address: khyey@kfri.re.kr (H.Y. Kim).

0378-8741/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2008.08.006

K_{ATP} channel such as the drug diazoxide (Ashcroft and Gribble, 1999).

Kimura et al. (1981) reported that some fractions extracted from KWG stimulated glucose-induced insulin release from pancreatic islet as a potentiator. Glucose-dependent insulin secretion is modulated by several hormones and neurotransmitters, among which acetylcholine (ACh) plays a prominent role (Gilon and Henquin, 2001). An important feature of the cholinergic regulation of pancreatic insulin release is that ACh stimulates insulin release in a glucose-dependent manner (Gilon and Henquin, 2001). Su et al. (2007) reported that KWG increased the release of acetylcholine from nerve terminals in rats so as to stimulate muscarinic M3 receptors activity located in the pancreatic cells for the secretion of insulin, which in turn lower plasma glucose.

Studies have reported that the biological activities of KWG are changed by heat processing (Kim et al., 2000). The changes in the biological activities of KRG were thought to be mediated by the changes in the chemical constituents such as ginsenosides by heat processing because ginsenosides are known as pharmacologically main active components of ginseng (Park et al., 1998). The insulinotropic activity of KWG has been reported, but the activity of heat-processed ginseng (KWG) are not yet fully elucidated. Therefore, the effect and mechanism of KRG on stimulation of insulin release was investigated using isolated rat islets.

2. Materials and methods

2.1. Ginseng extract

Korean red ginseng (KRG) was purchased in dried forms from Korea Ginseng Cooperation (Daejeon, Korea). The roots were *Panax ginseng*-strained, 6-year-old, cultivated in Korea, and straight-shaped in dried forms. The dried weight of one root was 20 g. The powdered ginseng (100 g) was extracted with ethanol/water (80/20, v/v) at a ratio of 20 ml/g for 5 h at reflux three times, filtered with filter papers (Toyo No. 2 and 4, Advantec, Japan). The combined filtrate was concentrated in a rotary vacuum evaporator ($T < 40\,^{\circ}C$) until half of the filtrate was removed, and freeze-dried so that solid residue was obtained. The residue afforded aqueous ethanolic extract (AEE) of KRG (30–35 g; yield, 30–35%).

2.2. Ginsenoside analysis

To analyze the ginsenoside profile, high-performance liquid chromatography (HPLC) was performed using the Jasco HPLC system (Tokyo, Japan) with a PU-2089 Plus gradient pump equipped with a degasser, a AS-2075 Plus autosampler, and a UV-2075 Plus UV–Vis detector. A Waters μBondapak C18 column (3.9 mm × 300 mm, i.d., 10 μm, Waters Corp., Milford, MA, USA) was used. The analysis was carried out with a binary mobile-phase gradient with a flow rate of 1.0 ml/min. Solvent A was water and solvent B was acetonitrile. The 70 min gradient method began with 80% A for 10 min, 68% A for 40 min, 57% A for 50 min, 20% A for 65 min, and 0% A for 70 min (Han et al., 2007). Before analysis, the extract was dissolved in 50% aqueous methanol to a 10 mg/ml concentration, and then passed through filters (0.45 μm, Millipore Corporation, Bedford, MA, USA). Ginsenosides Rb1, Rb2, Rb3, Rc, Rd, Re, Rf, Rg1, Rg2, Rg3, Rh1, and Rh2 were purchased from Fleton References Substance Co., Ltd. (Chengdu, China).

2.3. Islet isolation

The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the US guidelines (NIH publication #85-23, revised in 1985). The protocol for the care and use of animals was approved by the Institutional Care and Use Committee of the Korea Food Research Institute. The islets of Langerhans were isolated by injecting collagenase (Type IV, Sigma Chemical Co., MO, USA) in Hank's buffered saline solution (HBSS; 16.5 mg/15 ml) into the pancreas of male Sprague–Dawley rats (200–250 g body weight) through the pancreatic duct (Montague and Taylor, 1968). Then the gland was removed, incubated for 24 min at 37 °C, washed with HBSS, and islets were hand-picked up under a binocular dissecting microscope after separation on Ficoll gradient. Isolated islets were cultured overnight free floating in Petri dishes in Krebs–Ringer bicarbonate buffer (KRB; 1.2 mM $MgSO_4$, 4.7 mM KCl, 115 mM NaCl, 1.2 mM KH_2PO_4, 1.28 mM $CaCl_2$, 24 mM $NaHCO_3$, 10 mM HEPES-free acid, 0.1% BSA, pH 7.4) supplemented with 3.3 mM glucose and 10% fetal bovine serum at 37 °C at 95% air and 5% CO_2.

2.4. Static incubation experiments

After overnight equilibration, islets were washed and groups of three islets were incubated in the presence of AEE-KRG for 60 min at 37 °C in 1 ml of KRB containing glucose (3.3, 8.4, or 16.7 mM). AEE-KRG was dissolved in dimethyl sulfoxide (DMSO) to obtain desired concentrations. The final concentration of DMSO was 0.1%. After the 60-min incubations, the aliquots of the media were stored at −20 °C until insulin was measured. The islets were further incubated to elucidate the reversibility of the insulinotropic activity of AEE-KRG. After the islets were exposed to AEE-KRG (0.05–1.0 mg/ml) in the presence of glucose of 3.3, 8.4, or 16.7 mM for 60 min, the islets were washed with KRB and incubated in KRB for another 60 min. After the another 60-min incubations, the aliquots of the media were also stored at −20 °C until insulin was measured. Insulin was measured by an insulin immunoassay kit (Shibayagi Co., Japan) using rat insulin as a standard. Results were expressed as insulin released in the medium (ng/3 islets/60 min). Tolbutamide (Sigma Chemical Co.) was used as a positive control.

To study the effect of extracellular Ca^{2+} on insulin secretion induced by AEE-KRG, isolated islets were incubated in the absence or presence of AEE-KRG, under the following conditions of extracellular Ca^{2+}; in the presence of 1.28 mM Ca^{2+}; in the presence of 1.28 mM Ca^{2+} and the L-type Ca^{2+} channel blocker nifedipine (20 μM; "blocker"); in the absence of Ca^{2+} in the incubation medium; in the absence of Ca^{2+} but presence of the Ca^{2+} chelator EGTA (1 mM). Twenty micromolar of nifedipine was used according to the method of Chan et al. (1997). The contribution of K_{ATP} channel to insulin secretion induced by AEE-KRG was examined using islets pretreated with a K_{ATP} channel opener, diazoxide.

2.5. Statistical analysis

Experiments were performed in triplicate and replicated three or five times independently. All values were expressed as means and standard deviations (S.D.) or standard errors of means (S.E.M.). Student's *t*-test was used for statistical analyses (SAS software, SAS Institute, Cary, NC, USA). A *P* value of less than 0.05 was considered statistically significant.

3. Results

3.1. Ginsenoside analysis

The HPLC profile (Fig. 1) and content (Table 1) of ginsenosides (ginseng saponins) in KRG was shown. The contents in KRG of the ginsenosides Rg2, Rh1, Rg3, and Rh2, which are minor or absent in