Suppressive Effects of Polygonati Rhizoma on Hepatic Glucose Output, GLUT2 mRNA Expression and Its Protein Content in Rat Liver

ATSUSHI KATO, TOSHIHIRO MIURA, HIDEKI YANO*, KAZUHIRO MASUDA*, HITOSHI ISHIDA* and YUTAKA SEINO*

Kobe Women's College of Pharmacy, Hyogo 658, and *Department of Metabolism and Clinical Nutrition, Kyoto University School of Medicine, Kyoto 606, Japan

Abstract. The intraperitoneal administration of the methanol extract of Polygonati Rhizoma (OM) into normal rats caused a significant decrease in the blood glucose level at 4 h after its administration of 800 mg/kg (P<0.01), but not the serum insulin level. Using the perfused rat liver in vitro, a significant decrease of the hepatic glucose output was observed by the infusion of OM (P<0.05 at 250 µg/ml OM). In addition, the hepatic content of facilitative glucose transporter isoform 2 (GLUT2) mRNA and its protein content in the total membrane fraction from rat liver significantly decreased in the intraperitoneally OM-treated rats when compared to that in the controls (mRNA P<0.01, protein P<0.001). On the other hand, OM (500 µg/ml) exhibited no apparent stimulatory effect on the insulin secretion from the isolated rat pancreatic islets. These results suggest that the hypoglycemic effect of OM is derived, at least in part, from the decrease in hepatic glucose output, due presumably to the reduction of GLUT2 mRNA expression and its protein content in total membrane of the liver, and that because of its unique therapeutic mechanism, OM could be a new category of therapeutic agent for non-insulin-dependent diabetes mellitus.

Key words: Hypoglycemic activity, Polygonati Rhizoma, Hepatic glucose output, GLUT2, Northern blotting, Western blotting.

INCREASED hepatic glucose output is known as one of the major pathogenic factors of non-insulin-dependent diabetes mellitus (NIDDM), together with the insulin resistance in peripheral tissues and the impairment of glucose-induced insulin secretion from pancreatic β cells. Although the therapeutic agents to stimulate insulin secretion (for example, sulfonylureas) have been used for NIDDM patients, drugs to decrease hepatic glucose output are directly not yet available clinically, but they may be expected to become a new category of drugs which can be used in combination with the sulfonylureas and insulin because of the different therapeutic mechanisms.

Polygonati Rhizoma, "Ousei" in the Japanese nomenclature of Oriental medicine, and rhizomes of Polygonatum falcatum A. GRAY and the same genus plants, has been primarily used for the treatment of patients with diabetes-like symptoms (polyuria and polydipsia) in oriental traditional medicine. It has been found recently by us that the methanol extract of the root of this plant exhibits a significant hypoglycemic effect after intraperitoneal administration in normal and diabetic mice [1]. Furthermore, one of the active compounds of OM has been identified as a spirostanol glycoside

Received: July 9, 1993
Accepted: December 4, 1993
Correspondence to: Dr. Toshihiro MIURA, Dept. of Metabolism and Clinical Nutrition, Kyoto University School of Medicine, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606, Japan
(PO-2). However, the precise mechanism of its hypoglycemic effect has not been elucidated.

Glucose transport across the plasma membrane is mediated by carrier proteins termed glucose transporters [2, 3]. Recent cDNA cloning has demonstrated that the facilitative glucose transporters comprise a family of structurally related proteins with differing tissue distribution [4]. The gene expression and protein content of glucose transporters have been found to be altered under pathological conditions such as diabetes mellitus [4-6]. In the present study, we have examined the effect of Polygonati Rhizoma on hepatic glucose output, and we also have investigated the gene expression and protein content of the hepatic glucose transporter isoform 2 (GLUT2) in order to elucidate the mechanism of the hypoglycemic effect of OM and have found that OM decreases the hepatic glucose output and reduces both GLUT2 mRNA expression and its protein content in total membrane fraction from rat liver.

Materials and Methods

Materials

Ousei (Polygonati Rhizoma) which was obtained in the China market by Tochimoto Tenkaido Co., LTD. (Osaka, Japan), was used in the present experiment. Four kg of the rhizomes were extracted with two liters of methanol (65°C, 2 h, four times). The methanol extracts of these materials (OM) were lyophilized (183 g) and stored at 4°C until use. OM was dissolved in saline for the in vivo study and in buffers for the in vitro studies.

Animals

Male Wistar rats weighing 130–150 g were used for all studies. They were housed individually in an air-conditioned room at an ambient temperature of 24±2°C with a 12 h light-dark cycle. The rats were given laboratory chow and water ad libitum. OM was administered by intraperitoneal injections.

Hypoglycemic effect in fed rats

Blood samples were taken from the tail vein for the determination of blood glucose level. The hypoglycemic effect of OM was compared with that obtained by tolbutamide.

Five animals were used for each treatment group. Blood glucose levels were determined by the glucose oxidase method [7] and serum insulin was measured by radioimmunoassay using rat insulin (Novo Nordisk, Bagsvaerd, Denmark) as a standard [8].

Insulin secretion from rat pancreatic islets

Islets of Langerhans were isolated from male Wistar rats by collagenase digestion, as previously reported [9]. For the measurement of insulin secretion, isolated pancreatic islets (10–15 in each tube) were preincubated at 37°C for 30 min in Krebs-Ringer bicarbonate-HEPES buffer (KRHB) containing 129.4 mM NaCl, 5.2 mM KCl, 2.7 mM CaCl2, 1.3 mM KH2PO4, 1.3 mM MgSO4, 24.8 mM NaHCO3, 10 mM HEPES, 3.3 mM glucose, and 0.2% bovine serum albumin (BSA), pH 7.4 at 37°C. The islets were then incubated at 37°C for 30 min in KRHB with or without OM (500 µg/ml). Released insulin was measured by radioimmunoassay described above.

Hepatic glucose output from the perfused rat liver

Perfusion of the rat liver was started with Ca2+-free Hanks’ buffer which contained 1% BSA and 0.5 mM EGTA aerated with 95% O2/5% CO2 to pH 7.4 at 37°C. The flow rate of the perfusate was kept at 30 ml/min. After the liver had been perfused for 15 min for equilibration (preperfusion), the solution was changed with Hanks’ buffer contained various concentrations of OM. OM (50, 100, 250, 500 and 1000 µg/ml) was introduced into the portal vein for 20 min. Hepatic glucose output was measured from the glucose concentration of effluent perfusate obtained from the inferior vena cava 15 min after starting of preperfusion (prevalue) and 20 min after addition of OM. The decrease of glucose production was calculated by the following formula: (%) = [(prevalue - the value of OM-treated perfusion)/prevalue] × 100.

Northern blot analysis

The Wistar rats were given OM (800 mg/kg) intra-peritoneally, and the liver samples were obtained 4 h later.
HYPOGLYCEMIC EFFECT OF POLYGONATI RHIZOMA

Total RNA from liver and brain was freshly isolated by guanidium-thiocyanate-cesium chloride method [9]. Twenty micrograms of total RNA was denatured with glyoxal, electrophoresed on a 1% agarose gel, blotted onto a nylon membrane, and hybridized to nick-translated 32P-labeled rat GLUT2 cDNA probes. Hybridization was carried out at 42°C in a solution of 50% formamide, 5x sodium chloride sodium phosphate EDTA (SSPE) (1x SSPE=150 mM NaCl, 5 mM EDTA, and 50 mM sodium phosphate buffer, pH 7.4), 5x Denhardt's solution, 0.1% sodium dodecyl sulfate (SDS), and 100 µg/ml sonicated and denatured salmon testis DNA, and 10% dextran sulfate. After hybridization, the filters were washed three times in a solution of 0.1% sodium chloride-sodium citrate and 0.1% SDS for 5 min at room temperature, and then washed twice for 30 min each at 50°C. After autoradiography for different times to obtain a series of exposures, the relative level of mRNAs were quantified by densitometry of the autoradiograms.

Western blot analysis

The Wistar rats were administered OM (800 mg/kg) intraperitoneally and, 4 h later, the liver was resected for the experiment. The antibody used in the Western blotting was raised against a synthetic peptide corresponding to the COOH-terminal domain of rat GLUT2 (residues 498-522), as has been reported by Thorens et al. [10]. To prepare the total membrane particulate fractions, the rat livers were excised and 3-6 g of each liver slice was homogenized by a glass-Teflon homogenizer in 25 ml of 10 mM Tris-HCl, 1 mM EDTA, 250 mM sucrose, pH 7.4, containing 1 mM phenylmethyl sulphonyl fluoride and 1000 units/ml of aprotinin [11]. The homogenates were then centrifuged at 700 x g for 10 min at 4°C to sediment the fraction containing mainly the nuclei and mitochondria. The resulting supernatant was centrifuged at 146,000 x g for 75 min at 4°C to yield a pellet designated as the total membrane fraction of the liver in this study.

The total membrane fractions (0.1 mg) prepared were suspended in 1% SDS and 50 mM dithiothreitol and subjected to SDS-polyacrylamide (9%) gel electrophoresis. Electrophoretic transfer to nitrocellulose paper and detection of the immunocomplex with Enhanced Chemiluminescence (Amersham, Buckinghamshire, UK) were carried out as previously described [12]. The sheet was exposed on RX X-ray film and intensifying screen (Fuji, Tokyo, Japan). Prestained molecular weight standard (Bio-Rad, Richmond, VA, USA) was used for estimation of the molecular weight. The experiments were performed at least twice for each tissue with similar results.

Statistical analysis

All data are expressed as mean±SEM. ANOVA and Student's t test were used for the statistical analysis. Values were considered to be significantly different when P value was less than 0.05.

Results

Hypoglycemic effect in fed rats

The blood glucose levels at 4 h after the intraperitoneal administration of OM (200, 400 and 800 mg/kg) are presented in Fig. 1. These values are compared to those found when 0.9% saline was administered alone (control group) and also to those found when received tolbutamide (50 mg/kg body weight), a known hypoglycemic agent was administered. OM reduced blood glucose levels dose-dependently; significant decrease was observed with 800 mg/kg (P<0.01). Tolbutamide-treated rats also showed a significantly lower
blood glucose level at 4 h after the intraperitoneal administration ($P<0.05$). On the other hand, OM (800 mg/kg) exhibited no effect on serum insulin levels (38 ± 6 µU/ml in OM-treated rats and 32 ± 2 µU/ml in controls).

**Insulin secretion from isolated pancreatic islets**

As shown in Table 1, OM (500 µg/ml) tended to increase insulin secretion from the isolated pancreatic islets in the presence of 3.3 mM glucose, but there was no significant difference between OM-treated and control groups. In addition, no statistical difference was observed between the two groups even in the presence of 16.7 mM glucose.

**Hepatic glucose output from the perfused rat liver**

The dose-dependent suppressive effect of OM on hepatic glucose output from the perfused rat liver are shown in Fig. 2 ($P<0.05$ at 250 and 1000 µg/ml OM and $P<0.01$ at 500 µg/ml OM, respectively).

**Northern blot analysis**

Effects of OM on hepatic GLUT2 mRNA levels are shown in Fig. 3. Rat GLUT2 cDNA hybridized to 2.8 kilobases of mRNA from the liver of control and OM-treated rats under high stringent hybridization conditions. Densitometric scanning of the 2.8 kilobase bands revealed that the amount of liver GLUT2 mRNA in OM treated rats was reduced to 55% when compared to that in control rats ($P<0.01$). Expression of GLUT2 was not detected in brain, as has been previously reported [10].

**Western blot analysis**

The quantitation of GLUT2 protein in plasma membrane of rat liver was assessed by Western blotting in normal and OM-treated rats. Quantitation of the 55KDa GLUT2 glucose trans-
porter band isolated from nitrocellulose paper demonstrated that the relative amount of GLUT2 protein in the liver total membrane from OM-treated rats was only 20% of that observed in the control rats (P<0.001), as shown in Fig. 4.

**Discussion**

The present study clearly shows that the methanol extract of Polygonati Rhizoma (OM, 800 mg/kg) has significant hypoglycemic effects in normal rats. We have found further that OM (250 µg/ml) decreases the hepatic glucose output from the perfused rat liver. The circulating concentration of OM (800 mg/kg)-treated rats was estimated to be approximately 250 µg/ml by this finding. On the other hand, no effect was observed in the insulin secretion from the isolated pancreatic islets in vitro or serum insulin levels in vivo. From these results, it seems likely that OM exhibits its hypoglycemic effects mainly by decreasing the hepatic glucose output.

In order to elucidate the mechanism of the reduction of hepatic glucose output, we examined the effects of OM on GLUT2 glucose transporter in rat liver, since it has been reported that GLUT2 plays a crucial role in the process of glucose output and intake of the liver [4, 13]. The GLUT2 mRNA expression and its protein content were similarly decreased in the liver of the OM-treated rats. The decreased GLUT2 protein in the total membrane fraction is probably due to the decreased GLUT2 protein synthesis, since GLUT2 mRNA content also was observed to be suppressed. It has been previously demonstrated that GLUT2 mRNA and protein levels in the liver are significantly increased in streptozotocin-induced diabetic rats and it also has been suggested that the increased GLUT2 may contribute to the increased hepatic glucose output under the condition of insulin deficiency [11]. In addition, Yamamoto et al. also have shown that elevated GLUT2 mRNA expression in the liver may be associated with hyperglycemia and may be increased hepatic glucose production in Wistar fatty NIDDM rats [14].

Since we have shown previously that OM treatment decreases blood glucose levels in both normal and streptozotocin-induced diabetic mice [1], further study would justify how Ousei (Polygonati Rhizoma) could become a useful drug in the treatment of diabetes through this unique therapeutic mechanism.

**Fig. 4.** A: Effect of OM on GLUT2 protein content in rat liver. A: Western blot analysis of the membranes (50 µg) from the livers of control (C) and OM-treated rats (OM) with anti-peptide antibody directed against the COOH-terminal domain of rat GLUT2 glucose transporter. B: Quantitation of GLUT2 protein abundance in liver. Autoradiographic bands from hybridizations shown in Figure 4A were quantitated by densitometry. Each value represents the mean±SEM (N=4). Significantly different from control, *** P<0.001.
Acknowledgments

This study was partly supported by Grants-in-Aids for Scientific Research from the Ministry of Education, Science, and Culture and from the Research Committee of Experimental Models for Intractable Disease of the Ministry of Health and Welfare of Japan, and also by a grant for diabetic research from the Japan Diabetes Foundation and from Otsuka Pharmaceutical Company, Tokyo, Japan.

The authors thank Dr. D. Mayer for assistance in preparing the manuscript.

References