EFFECT OF GINSENG RADIX ON GLUT2 PROTEIN CONTENT IN MOUSE LIVER IN NORMAL AND EPINEPHRINE-INDUCED HYPERGLYCEMIC MICE

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The oral administration of the water extract of Ginseng Radix (GR) to normal and epinephrine-induced hyperglycemic mice caused a significant decrease in the blood glucose level 4 h after its administration. The hepatic content of facilitative glucose transporter isoform 2, liver type glucose transporter (GLUT2) protein content from mouse liver significantly increased in the orally GR-treated normal and epinephrine-induced hyperglycemic mice compared to that in the controls. These results suggest that the hypoglycemic activity of GR is presumably due, at least in part, to the increment of GLUT2 protein content.

KEY WORDS Ginseng Radix, hypoglycemic activity, GLUT2, Western blotting

Serum glucose concentrations are maintained by a balance between clearance of glucose by peripheral tissues and release of glucose into blood. The liver plays an especially important role in regulating these concentrations, because it serves as a center for distribution of glucose by taking up incoming glucose from the small intestine and releasing glucose into the circulation [1]. Both uptake and release of glucose require transport of glucose across the plasma membrane. In general, glucose transport across the plasma membrane is mediated by specific carriers termed glucose transporters [2], and the presence of a facilitative glucose transporter, GLUT2, was suggested in the liver [3].

Ginseng Radix, the root of Panax Ginseng C. A. Meyer (Araliaceae), has been primarily used as a tonic and nourishment in traditional oriental medicine. The water extract of Ginseng Radix (GR) exhibits a significatnt hypoglycemic effect after intraperitoneal administration to normal and diabetic mice, [4] and has an effect on the liver glucose metabolism [5, 6]. In the present study, we examined the effect of GR on the hepatic glucose transporter in order to elucidate the effect of membrane of GR.

MATERIALS AND METHODS

The water extract of GR (Nagano, Japan) was generously donated by Tsumura Company, and was dissolved in distilled water.

Animals : Male mice (ddY, 5 weeks old, SLC, Japan) were kept in an experimental animal room for 7 days with free access to food and water. They were housed individually in an air-conditioned room at an ambient temperature of 24±2 °C with a 12 h light-dark cycle. GR was administered by oral injection.

Epinephrine-induced Hyperglycemic Mice The adult ddY mice were given GR orally, and 3 h later the epinephrine (0.6 mg/kg body weight) solution was also administered intraperitoneally. Blood samples were collected 1 h after the administration of epinephrine.

Detarmination of Blood Glucose

Blood samples were taken from the eye with a capillary to determine blood glucose level. Five animals were used for each treatment group. Blood glucose levels were determined by the glucose oxidase method [7].

Western Blot Analysis

The mice were given GR (200 mg/kg) orally and, 4 h later, the liver was resected for the experiment (normal and epinephrine-induced hyperglycemic mice). The antibody used in the Western blotting (East Acres, USA) was raised against a synthetic peptide corresponding to the COOH-terminal domain of rat GLUT2(residues 498-522), as reported by Thorens et al. [8] (No reaction against brain, fat, or muscle. Does not cross-react with GLUT1 or GLUT4 tested). To prepare the total membrane particulate fractions, the mice livers were excised and 1-2 g of each liver slice was homogenized in 25 ml of 10 mM Tris-HCl, 1 mM phenylmethyl sulphonyl fluoride and 1000

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units/ml of aprotinin [9]. The homogenates were then centrifuged at 700 g for 10 min at 4 °C to sediment the fraction containing mainly the nuclei and mitochondria. The resulting supernatant was centrifuged at 13,000 g for 20 min at 4 °C to yield a pellet designated as the membrane fraction of the liver in this study. The 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 [10]. 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. Student's *t* test was used for the statistical analysis. Values were considered to be significantly different when *p* value was less than 0.05.

RESULTS

GR (200, 400 mg/kg) significantly reduced blood glucose of normal mice 4 hour after the oral administration, while GR at 50 mg/kg did not show such a decrease compared with controls (control; 174±4, 50; 169±10, 200; 142±9 $p<0.05$, 400; 126±10 mg/dl $p<0.05$) . However, the decrease of blood glucose in normal mice was slightly. In epinephrine-induced hyperglycemic mice, GR(200, 400 mg/kg) also reduced blood glucose under similar conditions (control; 399±22, 50; 429±14, 200; 307±25 $p<0.05$, 400; 255±24 mg/dl $p<0.01$)

Effects of GR(200 mg/kg ; 4 hours after administration) on hepatic GLUT2 protein levels in normal mice are shown in Fig. 1. Mouse GLUT2 antibody hybridized to 55 kilodalton of protein from the liver of control and GR-treated mice. Densitometric scanning of the 55 kilodalton bands revealed that the amount of liver GLUT2 protein in GR-treated mice (200 mg/kg) was increased to 212 % compared to that in normal mice. ($p<0.05$). The GLUT2 protein content in GR-treated epinephrine-induced hyperglycemic mice also was significantly increased to 267 % over that in control mice (Fig. 2) ($p<0.05$).

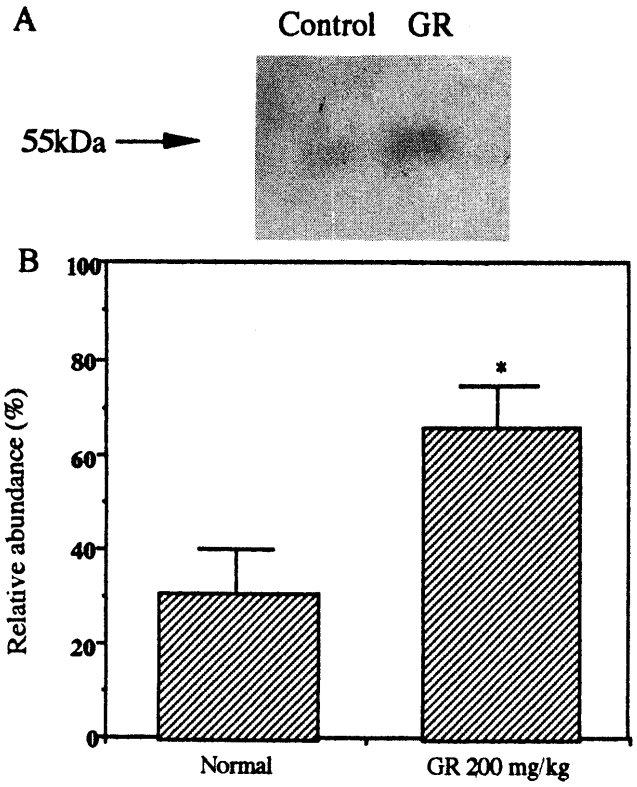

Fig. 1. Effect of GR on GLUT2 Protein in Normal Mice
Each value represents the mean±S. E. from 3 mice.
Significantly different from normal, *$p<0.05$.
A; photo, B; relative abundance by densitometry

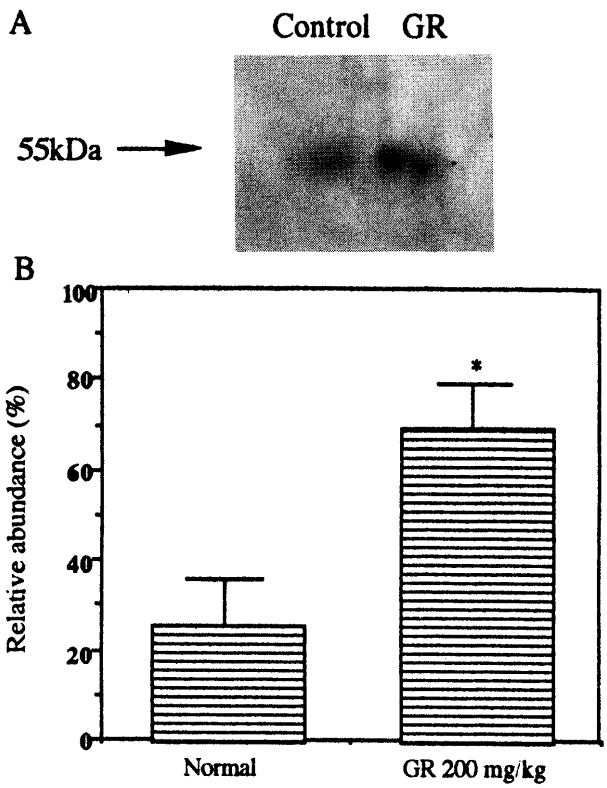

Fig. 2. Effect of GR on GLUT2 Protein in Epinephrine-induced Hyperglycemic Mice
Each value represents the mean±S. E. from 4 mice.
Significantly different from control, *$p<0.05$.
A; photo, B; relative abundance by densitometry

DISCUSSION

The present study clearly shows that the water extract of Ginseng Radix has a significant hypoglycemic effect on normal and epinephrine-induced hyperglycemic mice. In the basic study, we examined time-dependence (0, 4, 7h) after the treatment of GR, and found that GR had a hypoglycemic effect at 4h. We examined the effects of GR on GLUT2 glucose transporter in mouse liver, since it has been reported that GLUT2 plays a crucial role in the liver process of glucose output and intake [11]. Yamamoto et al. also have shown that elevated GLUT2 mRNA expression in the liver may increase hepatic glucose production in Wistar fatty rats [12]. In epinephrine-induced hyperglycemic mice, the liver GLUT2 protein content was increased compared with that of normal mice (T. Miura, unpublished data). This suggests that the elevation of GLUT2 by epinephrine is due to the presence of a cAMP response element (CRE)[13] and/or to increase glucose production in liver. GR-treated mice had even grater increase of GLUT2 than epinephrine-induced hyperglycemic mice. Moreover, 0.6 mg/kg is the maximum dose of epinephrine in the blood glucose (data not shown). From these findings, it may be that the elevation of GLUT2 by GR is due to increased glucose uptake in liver. It is reported that ginsenoside Rb2 of GR increased liver glucokinase [6], suggesting that the hypoglycemic mechanism of GR is due to increase in the glucose transport and, thereafter, to activation of glucokinase. No differences in protein expression on SDS-PAGE were observed between control and GR-treated mice (data not shown).

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