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THE EFFECT OF GINSENOSIDE-Rb_2 ON NITROGEN BALANCE

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ABSTRACT.—In diabetic rats given ginsenoside-Rb_2 for 6 days, nitrogen balance was studied. Ginsenoside-Rb_2 was found to suppress the total urinary excretion of nitrogen, increase nitrogen retention in the body, and thereby improve nitrogen balance.

The extract from the roots of *Panax ginseng* C.A. Meyer (Araliaceae) has been used as a therapeutic agent for various diseases including hyperlipemia, atherosclerosis, hypertension, and diabetes mellitus. In our laboratory, the effect of ginseng has so far been studied from the viewpoint of biosynthesis of DNA, RNA, and protein and metabolism of carbohydrate and lipid (1–3). Further experiments using various isolated saponins demonstrated that ginsenoside-Rb_2, whose structure was identified by Sanada *et al.* (4), has a potent physiological action on carbohydrate and lipid metabolism in normal rats (5,6). In diabetic rats that had the major disorder in carbohydrate metabolism, significantly decreased blood glucose level was observed after continuous ip administration of ginsenoside-Rb_2 (7). The hypoglycemic action of the ginsenoside-Rb_2 has also been suggested by evidence of the promotion of the glycolytic system and lipid biosynthetic system, resulting in the increased triglyceride level in the adipose tissue (8–10). In addition, consecutive ip administration of ginsenoside-Rb_2 resulted in a decrease in the level of urea in the hepatic tissue, an increase in the levels of hepatic ribosomal RNA and membrane-bound ribosomes, and partial normalization of the level of free amino acids in the blood and hepatic tissue (11); these findings suggested suppressed production of urea and facilitated protein biosynthesis in the hepatic tissue, thus indicating an anabolic effect of ginsenoside-Rb_2. In the present study, the effect of ginsenoside-Rb_2 on nitrogen balance was investigated in diabetic rats as part of a research project on nitrogen metabolism.

The nitrogen content of the food, urine, and feces samples is shown in Table 1. In normal rats given the 18% casein diet, the level of ingested nitrogen was 181 mg/6 h, whereas in diabetic rats given the 18% casein diet, the corresponding level was 396 mg/6 h, showing a markedly high value in comparison with normal rats. In diabetic rats given ginsenoside-Rb_2 for 6 days, the ingested nitrogen was almost the same as in the control group. On the other hand, the urinary nitrogen level was about 3 times higher in diabetic control rats than in normal rats. In diabetic rats given ginsenoside-Rb_2, the level of urinary nitrogen was significantly decreased, being 25% lower than that in the control group. However, the fecal nitrogen level was almost the same in the control and ginsenoside-Rb_2-treated groups (14 mg/6 h vs. 13 mg/6 h). Thus, the change in absorbed nitrogen calculated on the basis of ingested and fecal nitrogen exhibited a slight increase of about 4% in the rats administered ginsenoside-Rb_2 when compared with the control value (Table 1), whereas administration of ginsenoside-Rb_2 significantly increased the level of retained nitrogen by 45% of the control value; the retained nitrogen value was increased from 181 mg/6 h to 263 mg/6 h ($p<0.05$).

TABLE 1. Effect of Ginsenoside-Rb_2 on Nitrogen Balance.[a]

	Non-diabetic rats	Diabetic rats Control	Diabetic rats Rb_2
Nitrogen intake (mg/6 h)	181 ± 7 (100)	396 ± 23 [219][b] (100)	406 ± 16 [224][b] (103)
Urinary Nitrogen (mg/6 h)	73 ± 8 (100)	218 ± 17 [299][b] (100)	163 ± 21 [223][b] (75)[c]
Fecal Nitrogen (mg/6 h)	7 ± 1 (100)	14 ± 1 [200][b] (100)	13 ± 1 [186][b] (93)
Absorbed Nitrogen (mg/6 h)	179 ± 7 (100)	387 ± 20 [216][b] (100)	402 ± 12 [225][b] (104)
Retained Nitrogen (mg/6 h)	84 ± 4 (100)	181 ± 24 [215][b] (100)	263 ± 33 [313][b] (145)[c]

[a]Figures in parentheses are percentages of the non-diabetic or diabetic control rats.
[b]Statistical significance $p<0.001$ vs. non-diabetic rats.
[c]Statistical significance $p<0.05$ vs. diabetic control rats.

For judging the protein nutritional status in the body, nitrogen balance is widely used. Because nitrogen is almost completely excreted into the urine, the quantity of nitrogen excreted from the body is measured in terms of urinary nitrogen. The quantity of nitrogen retained in the body is calculated from the quantities of ingested nitrogen and excreted nitrogen, and a value for nitrogen balance is thus obtained. In the present study, the level of urinary nitrogen excreted was 3 times higher in diabetic rats than in normal rats, indicating increased decomposition of somatic protein in diabetic rats. On the other hand, in comparison with normal rats, the ingestion of nitrogen was about 2.2 times greater in diabetic rats, but the excretion of nitrogen was increased to a greater extent, resulting in an inclination of the nitrogen balance toward the negative side. In contrast, when the ginsenoside-Rb_2 was given to rats for 6 consecutive days, the urinary excretion of nitrogen was significantly decreased, with a tendency for the nitrogen balance to improve toward the positive side. Corroborating this, the nitrogen retention was significantly increased in the ginsenoside-Rb_2 administration group, although there were no significant differences in nitrogen absorption between the control and ginsenoside-Rb_2-administered groups. Treatment with ginsenoside-Rb_2 resulted in increased availability of ingested protein.

Undersupply of glucose to muscle or other tissues due to insulin deficiency causes a decrease in the production of free energy, leading to energy supplementation through decomposition of protein, thus resulting in a decline in the nitrogen equilibrium to a negative value. It seems that the metabolic system in the rats after ginsenoside-Rb_2 administration was oriented in the direction of improvement in nitrogen balance.

EXPERIMENTAL

ANIMALS.—Male rats of the Wistar strain, weighing about 250 g, were employed in this experiment. Diabetes was induced in the rats by ip administration of streptozotocin (50 mg/kg body wt) dissolved in 10 mM citrate buffer (pH 4.5) (12). Two weeks after the injection, the blood glucose was determined, and rats with a glucose level of 380–410 mg/dl were used as diabetic rats. Before the study of nitrogen balance, all rats were housed in individual metabolic cages for 10 days and given an 18% casein diet composed of 18% casein, 57.9% α-cornstarch, 15% sucrose, 2%

soybean oil, 4% salt mixture (13), 1% vitamin mixture (13), 2% cellulose powder, 0.1% choline chloride, and H_2O ad libitum.

SAPONIN.—Ginsenoside-Rb_2 was isolated and purified from a root extract of *P. ginseng* produced in Kumsan, Korea. The structure of ginsenoside-Rb_2 was previously established by Sanada *et al.* (4) as (20*S*)-protopanaxadiol-3-[*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside]-20-[*O*-α-L-arabinopyranosyl(1→6)-β-D-glucopyranoside].

NITROGEN BALANCE EXPERIMENT.—The normal rats were fed an 18% casein diet. Diabetic rats were divided into 3 groups just before the study of nitrogen balance; two groups were given the same 18% casein diet and the other was given a nonprotein diet to estimate the endogenous nitrogen level. Ginsenoside-Rb_2 (10 mg/rat/day) dissolved in saline was administered ip to rats for 6 days, while control rats were treated with an equal volume of saline. For assay of nitrogen excretion, urine was collected for 6 h after the last ginsenoside-Rb_2 administration. Feces were also collected during the balance study and lyophilized. The food intake of each rat was recorded. The nitrogen content of the urine, feces, and food samples was analyzed by a TN-5 or TN-10 total nitrogen analyzer (Mitsubishi Chemical Industries Inc., Kanagawa, Japan). The nitrogen level is calculated on the basis of the standard curve obtained for a standard solution $(NH_4)_2SO_4$. Absorbed nitrogen is calculated on the basis of ingested and fecal nitrogen using the equation shown below. Retained nitrogen is estimated from the absorbed nitrogen and urinary nitrogen.

Absorbed nitrogen = ingested nitrogen − (fecal nitrogen after protein diet − fecal nitrogen after nonprotein diet).

Retained nitrogen = absorbed nitrogen − (urinary nitrogen after protein diet − urinary nitrogen after nonprotein diet).

STATISTICS.—Results were expressed as means ± SE of 6 rats. The significance of differences between the non-diabetic and diabetic rats (control or ginsenoside-Rb_2-treated group) was tested by Student's *t*-test.

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