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FACILITATION OF PROTEIN BIOSYNTHESIS BY GINSENOSIDE-Rb_2 ADMINISTRATION IN DIABETIC RATS

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ABSTRACT.—In streptozotocin-induced diabetic rats given 10 mg/day of ginsenoside-Rb_2 for 3 days, there was no significant increase in the serum protein and albumin levels, whereas a significant increase in these parameters was found in rats given the ginsenoside-Rb_2 for 6 days. When the state of protein biosynthesis was determined using [^{14}C] leucine, significantly activated protein biosynthesis was found in rats given ginsenoside-Rb_2 for 3 days, indicating the presence of an activated state of protein biosynthesis prior to the evident quantitative increase.

This laboratory has demonstrated that ginsenoside-Rb_2 partly restores the metabolic disorder characteristic of diabetes mellitus. Consecutive ip administration of ginsenoside-Rb_2 to diabetic rats resulted in an obvious, persistent decrease in blood glucose by increasing the channeling of metabolism into the glycolytic system, with evidence of improvement in diabetic symptoms such as body weight loss, polyphagia, polyposia, polyuria, and glucosuria (1–3). High blood urea nitrogen levels in diabetic rats were markedly decreased by ginsenoside-Rb_2 administration. The level of urea in hepatic tissue was also decreased. In addition, ginsenoside-Rb_2 resulted in an increase in the levels of hepatic ribosomal RNA and membrane-bound ribosomes. These findings suggested suppressed production of urea and facilitated protein biosynthesis in hepatic tissue (4,5). In the present study, the effects of ginsenoside-Rb_2 on protein biosynthesis in diabetic rats were investigated in detail.

RESULTS AND DISCUSSION

The serum constituents are shown in Table 1. Compared with that of nondiabetic rats, the level of total protein was markedly low in diabetic animals. Similar changes were seen in the albumin level. In diabetic rats given 10 mg/day of ginsenoside-Rb_2 for 3 consecutive days, the total protein level showed no appreciable changes. The rats in

TABLE 1. Effect of Ginsenoside-Rb_2 on Serum and Blood Constituents after 3 or 6 Days of Administration to Rats.[a]

Day	Group	Total protein (g/dl)	Albumin (g/dl)	Blood glucose (mg/dl)
+3	Nondiabetic	5.85 ± 0.14	3.56 ± 0.11	110.1 ± 3.2
	Diabetic			
	Control	5.06 ± 0.09[d]	3.09 ± 0.07[c]	396.7 ± 18.7[d]
	Rb^2	5.11 ± 0.18[c]	3.24 ± 0.11[b]	337.2 ± 22.1[d,e]
+6	Nondiabetic	5.95 ± 0.15	3.57 ± 0.16	117.4 ± 4.7
	Diabetic			
	Control	4.86 ± 0.30[c]	3.07 ± 0.12[b]	401.3 ± 10.7[d]
	Rb_2	5.68 ± 0.22[e]	3.39 ± 0.08[e]	321.0 ± 26.9[d,e]

[a]Values are means ± S.E. of 6 rats.
[b]$P<0.05$ vs. nondiabetic control.
[c]$P<0.01$ vs. nondiabetic control.
[d]$P<0.001$ vs. nondiabetic control.
[e]$P<0.05$ vs. diabetic control.

the group treated with ginsenoside-Rb_2 for 6 consecutive days showed a significant 17% increase in total protein. The data in Table 1 further indicate that the ginsenoside-Rb_2-treated group of diabetic rats showed a significant increase in the albumin level after 6 days of administration. In comparison with nondiabetic rats, diabetic rats exhibited a decrease in the incorporation of radioactive precursor into serum protein (Table 2). When ginsenoside-Rb_2 was given at a daily dose of 10 mg once a day, the rate of in-

TABLE 2. Effect of Ginsenoside-Rb_2 on the Incorporation of [^{14}C] Leucine into Serum Protein after 3 or 6 Days of Administration to Rats.[a]

Day	Group	Incorporated radioactivity (cpm/mg protein)
+3	Nondiabetic	829 ± 36
	Diabetic	
	Control	640 ± 50[b]
	Rb_2	921 ± 44[b,c]
+6	Nondiabetic	766 ± 33
	Diabetic	
	Control	612 ± 40[b]
	Rb_2	699 ± 43

[a]Values are means ± S.E. of 6 rats.
[b]$P<0.01$ vs. nondiabetic control.
[c]$P<0.001$ vs. diabetic control.

corporation of labeled precursors into serum protein was enhanced after 3 days of administration (significantly increased by 44% of the control value). The data in Table 2 further indicate that the incorporation of radioactive precursor was about 14% higher at the 6th day in the ginsenoside-Rb_2-treated group as compared with the control group (this variation was not statistically significant). On the other hand, the incorporation of labeled precursor into subcellular fractions in diabetic rats on the 3rd or 6th day exhibited a significant decrease in the homogenate, nuclear, and microsomal fractions, while the mitochondrial and soluble fractions showed no appreciable changes. As a result of ginsenoside-Rb_2 administration for 3 days, the incorporation of labeled amino acid was significantly increased in the homogenate, nuclear, mitochondrial, and microsomal fractions, excluding the soluble fraction. These levels were 20%, 8%, 13%, and 24% higher, respectively, than the corresponding levels in the control group. Significantly increased incorporation of labeled amino acid in the homogenate, nuclear, and microsomal fractions was observed after 6 days of administration of ginsenoside-Rb_2. In particular, the incorporation of radioactive precursor into microsomal protein was extremely high, being 48% higher than that of the control value. A moderate increase was found in the mitochondrial and soluble fractions, but this variation was not statistically significant (Table 3).

It is known that the metabolic disorder induced by diabetes mellitus is reflected not only in carbohydrate and lipid metabolism but also in protein metabolism. In diabetes mellitus, the metabolic pattern in the body comes to resemble that seen in starvation, despite the fact that food is ingested. Therefore, the starvation-type regulation mechanism, which would normally act to maintain the homeostasis of the body, conversely gives rise to a vicious cycle (6).

In the present study, in order to investigate the effects of ginsenoside-Rb_2 on protein biosynthesis in relation to the period of administration, 10 mg of the saponin was given to rats for 3 or 6 consecutive days. In rats given ginsenoside-Rb_2 for 3 days, there

TABLE 3. Effect of Ginsenoside-Rb_2 on the Incorporation of [^{14}C] Leucine into Hepatic Subcellular Fractions after 3 or 6 Days of Administration to Rats.[a]

Day	Group	Incorporated radioactivity (cpm/mg protein)				
		Homogenate	Nuclear fr.	Mitochondrial fr.	Microsomal fr.	Soluble fr.
+3 . . .	Nondiabetic	457 ± 13	328 ± 8	412 ± 18	643 ± 16	276 ± 4
	Diabetic					
	Control	401 ± 16[b]	272 ± 8[d]	377 ± 10	555 ± 33[b]	265 ± 8
	Rb_2	481 ± 25[e]	294 ± 8[b,e]	426 ± 9[f]	689 ± 29[f]	287 ± 10
+6 . . .	Nondiabetic	417 ± 15	351 ± 18	385 ± 11	655 ± 18	251 ± 9
	Diabetic					
	Control	372 ± 3[b]	273 ± 8[c]	357 ± 14	499 ± 57[b]	227 ± 8
	Rb_2	424 ± 19[e]	314 ± 13[e]	395 ± 18	737 ± 52[f]	242 ± 12

[a]Values are means ± S.E. of 6 rats.
[b]$P<0.05$ vs. nondiabetic control.
[c]$P<0.01$ vs. nondiabetic control.
[d]$P<0.001$ vs. nondiabetic control.
[e]$P<0.05$ vs. diabetic control.
[f]$P<0.01$ vs. diabetic control.

was no significant increase in the serum total protein level, whereas a significant increase in this parameter was found in rats given the ginsenoside-Rb_2 for 6 days. Similarly, the group treated with ginsenoside-Rb_2 for 6 days showed a significant increase in albumin, which is synthesized in and secreted from the liver (7). When the state of protein biosynthesis was determined using [^{14}C] leucine, significantly activated protein biosynthesis was found in rats given ginsenoside-Rb_2 for 3 days, indicating the presence of an activated state of protein biosynthesis prior to the evident quantitative increase. Furthermore, determination of the distribution of [^{14}C] leucine in subcellular fractions prepared from the liver revealed a high activity, excluding the soluble fraction. Significantly increased incorporation into the microsomal fraction was observed in rats given ginsenoside-Rb_2 for 3 or 6 consecutive days. On the basis of the increased incorporation of [^{14}C] leucine by the hepatic microsomal fraction and the previously reported increase in the quantity of membrane-bound ribosomes, the increase in the serum protein content was clearly attributable to an increase of secretory protein biosynthesis induced by ginsenoside-Rb_2.

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 is considered that ginsenoside-Rb_2 converts this type of metabolic pattern to an anabolic one. Corroborating this, 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 (8). In other words, ginsenoside-Rb_2 seems to act as a type of metabolic modulator.

EXPERIMENTAL

ANIMALS AND TREATMENTS.—Male rats of the Wistar strain, ranging in weight from 200 to 210 g, were employed in this experiment. Diabetes was induced in the rats by ip administration of streptozotocin (50 mg/kg) dissolved in 10 mM citrate buffer (pH 4.5) (9). Two weeks after injection, blood glucose was determined, and rats with a glucose level of 390–420 mg/dl were used as diabetic rats. Ginsenoside-Rb_2 (10 mg/rat/day) dissolved in saline was administered ip to rats for 3 or 6 days, while control rats were treated with an equal volume of saline. Six h after the last treatment, rats were sacrificed by means of a sharp blow to the back of the head and exsanguinated. The blood was collected in a conical centrifuge tube, and

the serum was immediately separated by centrifugation. The liver was removed quickly and placed in liquid nitrogen. The blood glucose level of the ginsenoside-Rb_2-administered rats used in this experiment showed a significant decrease as compared to the control group. By the 3rd day in the ginsenoside-Rb_2-treated group, the blood glucose level had fallen significantly, by 15%, compared with the control value (Table 1). Six days of ginsenoside-Rb_2 administration resulted in a further decrease.

SAPONIN.—Ginsenoside-Rb_2 was isolated and purified from a root extract of *Panax ginseng* C.A. Meyer produced in Kumsan, Korea, and a voucher specimen deposited in the Herbarium of Toyama Medical and Pharmaceutical University, Toyama, Japan. The structure of ginsenoside-Rb_2 has been previously established by Sanada *et al.* (10) as (20*S*)-protopanaxadiol-3-[*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside]-20-[*O*-α-L-arabinopyranosyl(1→6)-β-D-glucopyranoside].

CHEMICALS.—Streptozotocin was purchased from Sigma Chemical, USA. [^{14}C-U-L] Leucine was a product of New England Nuclear, USA. ACS II scintillation cocktail was supplied by Amersham, USA. All other chemicals used in this study were of analytical or reagent grade.

STATISTICS.—The significance of differences between the nondiabetic and diabetic rats (control or ginsenoside-Rb_2-treated group) was tested by Student's *t*-test.

DETERMINATION OF SERUM CONSTITUENTS.—Total protein and albumin were determined by the biuret method (11) and BCG method (12), respectively.

DETERMINATION OF RADIOACTIVITY INCORPORATED INTO SERUM PROTEIN.—Five h after the last administration of ginsenoside-Rb_2, the rats received [^{14}C] leucine (4 μCi/rat) in 0.5 ml of saline ip, and blood samples were collected 1 h later. The sera obtained by centrifugation were treated with perchloric acid (PCA) (final concentration, 5%). The PCA-insoluble residues were washed with 5% PCA followed by Me_2CO and Et_2O. The samples were then air-dried. The dry powder was dissolved in 0.1 N NaOH, and aliquots were used for protein determination by Lowry's method (13), with bovine serum albumin as a standard. The radioactivity was determined in an Aloka liquid scintillation spectrometer, model LSC-900, after the aliquots had been suspended in ACS II liquid scintillation cocktail. The specific radioactivity was estimated as cpm/mg protein from the radioactivity and colorimetric determinations.

DETERMINATION OF RADIOACTIVITY INCORPORATED INTO HEPATIC SUBCELLULAR FRACTIONS.—Subcellular fractions were prepared by a modification of the method of Fleischer and Kervina (14). The liver, after 1-h [^{14}C] leucine (4 μCi/rat) in vivo labeling, was homogenized with 4 volumes of chilled 0.25 M sucrose in TKM buffer (50 mM Tris-HCl, 25 mM KCl, 5 mM $MgCl_2$, pH 7.5) in a Potter-Elvehjem device with 10 strokes of a motor-drived Teflon pestle. The homogenate was filtered through 4 layers of gauze, and the filtered fraction was taken as the liver homogenate fraction. This fraction was centrifuged successively at 1000 *g* for 10 min, at 9500 *g* for 10 min, and at 105,000 *g* for 60 min. The resulting precipitates from individual centrifugations were taken as the nuclear fraction, mitochondrial fraction, and microsomal fraction, respectively. These fractions were each dissolved in chilled distilled H_2O. The supernatant fraction resulting from the centrifugation at 105,000 *g* for 60 min was taken as the soluble fraction. Specific radioactivities in these fractions were determined by the same procedure as that described above.

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