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Effects of ginsenoside-Rb_2 on adenine nucleotide content of rat hepatic tissue

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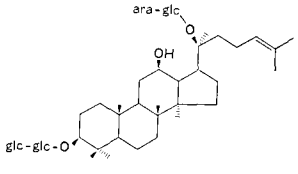
Abstract—In comparison with non-diabetic rats, the tissue ATP content of diabetic rats is lower and the AMP content higher. However, daily intraperitoneal administration of ginsenoside-Rb_2 for several days resulted in an increase of tissue ATP content and a decrease of AMP content, characterized by an increase in the adenine nucleotide content, as well as improvement of energy charge. Thus, ginsenoside-Rb_2 administration caused a change in the pattern of metabolism, activating the ATP supply system.

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 of the body comes to resemble that seen in starvation, due to insulin deficiency, despite the fact that food is ingested. Therefore, the starvation-type regulation mechanism, which would normally act to maintain homeostasis, conversely gives rise to a vicious cycle (Bondy 1971).

We have previously demonstrated in rats that ginsenoside-Rb_2 (I) partly restores the metabolic disorder induced by diabetes mellitus (Yokozawa et al 1985a, b, 1987a, b). Our data indicated that the metabolic system in the rats after ginsenoside-Rb_2 administration was oriented in the direction of energy production.

Among many high-energy phosphate compounds involved in metabolic routes in the body, the adenine nucleotide series plays a role in the metabolism of carbohydrate, lipid and protein. In the present study, to investigate the effects of ginsenoside-Rb_2 on the energy production system, we administered ginsenoside-Rb_2 to diabetic rats and determined ATP, ADP and AMP in hepatic tissue.



I. Structural formula of ginsenoside-Rb_2.

Materials and methods

Animals and treatments. Diabetes was induced in male Wistar rats, 200 g, by intraperitoneal administration of streptozocin (50 mg kg^{-1}) dissolved in 10 mM citrate buffer (pH 4·5) (Junod et al 1967). Two weeks after the injection, the blood glucose was determined, and rats with a glucose level of 390–420 mg dL^{-1} were used as diabetic rats. Ginsenoside-Rb_2 (10 mg/rat/day) dissolved in 0·9% NaCl (saline) was administered intraperitoneally to rats for 3 or 6 days, while control rats were treated with an equal volume of saline. At 6 h after the last treatment, rats were decapitated.

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Saponin. Ginsenoside-Rb_2 (I) was isolated and purified from a root extract of *Panax ginseng* C. A. Meyer produced in Kumsan, Korea, according to Sanada et al (1974).

Extraction of adenine nucleotide. A portion of excised liver (ca 300 mg) was immediately frozen using a freeze-clamp precooled with liquid nitrogen. The cutting to freezing time was less than 3 s. Extraction of adenine nucleotide was in a cold room using a slight modification of the method of Chen et al (1977). The frozen tissue was powdered in a porcelain mortar while liquid nitrogen was continually added. Fifteen volumes of 0·5 M perchloric acid was added in the same mortar. The powdered mixture was homogenized by 10 strokes in a Potter-Elvehjem homogenizer and centrifuged at 3000 rev min^{-1} for 5 min. The supernatant obtained was neutralized with 5 M potassium hydroxide. The resulting precipitate was removed by centrifugation and the supernatant was used for analysis of adenine nucleotide. In this extraction method, recovery of ATP, ADP and AMP was more than 95%.

Measurement of adenine nucleotide. A Shimadzu LC-6A high-performance liquid chromatograph (Shimadzu Co., Kyoto, Japan) connected to an integrator, with a double-beam UV detector operating at a wavelength of 260 nm, was used. The gradient accessory consisted of an electronic programmer and a second pump. Adenine nucleotide was eluted with 20 mM phosphate buffer (pH 7·0) and 480 mM phosphate buffer (pH 6·9) by the linear gradient method using a Shim-pack WAX-1 column. The flow rate was 1 mL min^{-1} and the temperature of the column was 45°C. Each peak in the chromatogram was identified by comparison with the retention time of authentic compounds. Adenine nucleotide was further confirmed by the addition of authentic nucleotide to the sample. The energy charge was calculated according to Atkinson (1975).

$$\text{Energy charge} = \frac{\text{ATP} + 1/2\text{ADP}}{\text{ATP} + \text{ADP} + \text{AMP}}$$

Statistics. Results were expressed as means ± s.e. for six 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.

Results

The blood glucose level of the ginsenoside-Rb_2-administered rats used in this experiment showed a significant decrease compared with the diabetic control rats (Table 1). Changes in adenine nucleotide contents are also shown in Table 1. In diabetic rats given ginsenoside-Rb_2 for either 3 or 6 days the energy charge was increased significantly (Table 1).

Discussion

It has been demonstrated that the nitrogen balance shows a negative tendency in rats with diabetes induced by streptozocin (hypoproteinaemia, hypoalbuminaemia, increased blood urea nitrogen, increased urea in hepatic tissue, decreased RNA, decreased serum protein synthesis and increased urinary excre-

Table 1. Blood glucose, hepatic adenine nucleotide content and energy charge.

Parameters	Non-diabetic rats	Diabetic rats		
		Control	Ginsenoside-Rb_2 3 days	Ginsenoside-Rb_2 6 days
Blood glucose (mg dL^{-1})	115·4 ± 4·0	386·6 ± 16·8***	328·6 ± 20·8***,a	301·5 ± 22·5***,b
ATP (μmol (g tissue)$^{-1}$)	5·15 ± 0·21	3·84 ± 0·08***	4·54 ± 0·05*,b	5·16 ± 0·17c
ADP (μmol (g tissue)$^{-1}$)	1·38 ± 0·10	1·36 ± 0·04	1·14 ± 0·07*,b	1·41 ± 0·02
AMP (μmol (g tissue)$^{-1}$)	0·28 ± 0·04	0·65 ± 0·14*	0·47 ± 0·02***	0·35 ± 0·01*,a
Total adenine nucleotide (μmol (g tissue)$^{-1}$)	6·80 ± 0·08	5·85 ± 0·10***	6·14 ± 0·11**,a	6·93 ± 0·15c
Energy charge	0·86 ± 0·01	0·77 ± 0·02**	0·83 ± 0·05a	0·85 ± 0·05b

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$ vs non-diabetic rats, a $P < 0·05$, b $P < 0·01$, c $P < 0·001$ vs diabetic control rats.

tion of nitrogen) (Jefferson et al 1983). In the present study, in comparison with non-diabetic rats, such diabetic rats were found to have decreased ATP and increased AMP in hepatic tissue, with decreased adenine nucleotide and energy charge, showing a malnutritional state due to insufficient supply of ATP. On the basis of these findings, the decrease in the total adenine nucleotide level in diabetic rats was considered to be attributable to enhanced catabolism of purine bodies. When diabetic rats having a lower ATP level in hepatic tissue than that in non-diabetic rats were given 10 mg ginsenoside-Rb_2 intraperitoneally once daily, the decreased level was restored almost to normal after six days of administration. On the other hand, there was a decrease in the AMP level, which had been increased by the diabetic condition. An increase in adenine nucleotide was also noted. These results suggest that ginsenoside-Rb_2 restores the balance of energy metabolism in the diabetic condition. This idea was also supported by the inclination of the energy charge towards the positive side. However, there arises a question as to whether the increased ATP level observed in rats given ginsenoside-Rb_2 was caused by activation of the ATP supply system or by decreased utilization of ATP. As reported previously, rats given ginsenoside-Rb_2 showed a low blood glucose level, a high glucokinase/glucose-6-phosphatase activity ratio, increased glycolysis, lipogenesis and serum protein synthesis, a high quantity of hepatic ribosomes, and increased synthesis of protein in the hepatic microsomal fraction (Yokozawa et al 1985a, 1987a, b; Oura 1988). Nitrogen retention in the body was significantly increased in rats given ginsenoside-Rb_2 (Yokozawa et al 1989a, b). Since metabolism in these rats was activated in the direction of somatic protein synthesis along with improvement in the nitrogen balance, it was considered that ATP production was increased, exceeding the level compensating for ATP consumption, and therefore inducing an increase in the ATP level in hepatic tissue. On the other hand, non-diabetic rats did not show any of the ginsenoside-Rb_2 effects seen in diabetic rats. In cases of diabetes, the body shows the starvation-type metabolic pattern due to insulin deficiency. In this condition, ginsenoside-Rb_2 seems to exert its action to restore normal metabolism, whereas it has no effects on non-diabetic rats. Since no changes in the serum insulin level have been seen in diabetic rats after ginsenoside-Rb_2 administration (Yokozawa et al 1987b), it remains unclear whether ginsenoside-Rb_2 serves as a modifier which increases the sensitivity to insulin, enhancing the action of insulin, or inhibits the secretion of ACTH or adrenaline, causing the inhibition of the anti-insulin action.

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