Mechanism of the enhancing action of rhatannin on hepatic glutamine transamination in rats

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Abstract

The effect of rhatannin on hepatic glutamine transamination was determined in rats under the following dietary conditions : protein feeding, carbohydrate feeding, and fasting. Glutamine transamination activity was estimated by measuring the glycine formation rate from glutamine and glyoxylate. Rhatannin caused liver glutamine transamination to increase in carbohydrate-restricted rats. In addition, glucagon and N^6, $O^{2'}$-dibutyryl cyclic adenosine-3′,5′-monophosphate (DBcAMP) shared the increase of the transamination under the present experimental condition. The increased transamination caused by rhatannin resulted from the increase of glutamine-glyoxylate transamination but not increases of glutamate-glyoxylate and albizziin-glyoxylate transaminations. This enhanced glutamine transamination activity existed in rat liver mitochondrial fraction and was abolished by cortisone treatment. These metabolic responses induced by rhatannin were similar to those of glucagon. The present experiments suggested that the increased glutamine transamination might be accompanied with an increased gluconeogenesis and the positive effect of rhatannin on this transamination might be mediated through its action on the glucagon regulatory pathway.

Key words rhatannin, Rhei Rhizoma, condensed tannin, glutamine metabolism, rat liver, rat liver mitochondria, glucagon

Abbreviations DBcAMP, N^6, $O^{2'}$-dibutyryl cyclic adenosine -3′, 5′- monophosphate ; EDTA, ethylenediaminetetraacetate

Introduction

There are accumulating data dealing with chemical and biological studies on tannin. A substance with blood urea-nitrogen decreasing activity was extracted and partially purified from Rhei Rhizoma which belongs to a category of tannin and was designated as rhatannin.[1,2] In our previous studies, it was demonstrated that rhatannin had influences on hepatic amino acid metabolism, *e.g.*, a decrease of urea production,[3] increases of amino acid and ammonia uptake,[3] an increase of protein synthesis,[4] and increases of glutamine synthetase activity[3] and glutamine transamination.[5]

It is well known that these parameters are under hormonal control. Our previous data, with the use of rat hepatocytes, showed that the presence of glucagon was required for the expression of enhancing action of rhatannin on gluconeogenesis from amino acid.[6] The results can be interpreted as suggesting that the effect of rhatannin on this process might be mediated through its action on the glucagon regulatory pathway.

Since it has been demonstrated that glucagon

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stimulates the liver transamination between glutamine and glyoxylate without affecting the transaminations between either glutamate or albizziin and glyoxylate,[7-11] the present experiment was designed to explore the interrelationships of the action of rhatannin and glucagon with the level of glutamine transamination activity. It was shown that rhatannin caused the glutamine transamination to enhance only in carbohydrate-restricted animals. The results suggested that the increase of the transamination might be associated with a promotion of gluconeogenesis and that the mechanism responsible for the effect of rhatannin might be mediated through its action on the glucagon regulatory pathway.

Materials and Methods

Materials : Rhatannin was isolated from Rhei Rhizoma, *Rheum officinale* BAILLON (Sichuan, 四川 ; China), Gaô (Jia-Huang, 雅黃), by the method described previously.[2] The following compounds were purchased : sodium glyoxylate, L-α-amino-β-ureidopropionic acid (albizziin), N^6, $O^{2'}$-dibutyryl cyclic adenosine-3',5'-monophosphate (DBcAMP) (Sigma Chemical Co., U.S.A) ; glucagon (Novo Pharmaceutical Ind., Denmark) ; glutamine, sodium glutamate (Wako Pure Chem. Ind., Japan) ; disodium ethylenediaminetetraacetate (EDTA) (Dojin Laboratories, Japan).

Animals and Diets : Male Wistar rats weighing 160−180 g were employed throughout the experiment. They were housed in air-conditioned quarters at 25℃ and 60% relative humidity. Animals were fed on laboratory pellet chow (CE-2, CLEA Japan Inc., Japan) and tap water freely. For fasting, rats were separated from the chow for 24 hr or 72 hr. Those rats deprived of chow lost 14.4±3.8 g S.D. body weight/24 hr or 34.8±3.8 g / 72 hr, respectively. For meal feeding, rats were fasted overnight on the 1st day and then fed either a protein-rich and carbohydrate-free diet or a protein-free and carbohydrate-rich diet for 3 days. The former contained 70% casein(Oriental Yeast Co., Ltd., Japan), 22.9% cellulose powder (CF1, Whatman Paper Ltd., England), 2% soybean oil (CLEA Japan Inc.), 4% salt mixture (Oriental Yeast Co., Ltd.), 1% vitamin mixture (Oriental Yeast Co., Ltd.) and 0.1% choline chloride (Wako Pure Chem. Ind.). In the latter diet, α-cornstarch (CLEA Japan) was substituted for casein. Those meal fed rats gained 10.9±5.6 g S.D. body weight/3 days in the former diet and lost 20.7±5.8 g/3 days in the latter, respectively. Rhatannin (12.5 mg/kg body weight) was administered intraperitoneally to rats, and control rats were given an equal volume of saline. Glucagon (1 mg/kg), DBcAMP (30 mg/kg), when used, were administered to rats. Cortisone acetate (30 mg/kg) and cycloheximide (3 mg/kg) were injected to rats 30 min prior to rhatannin, glucagon, or saline treatment. All animals were sacrificed 4 hr after the treatment.

Preparation of liver extract : Each animal was killed by decapitation, and the liver was removed and disrupted with polytron homogenizer at full speed for 30 sec in 3 vol 10 mM potassium phosphate buffer (pH 7.2), followed by freeze thawing. The homogenate was centrifuged at 15,000 ×g for 20 min and the supernate was assayed to determine the glycine formed.

Subcellular fractionation of rat liver : Rat livers removed immediately after decapitation were minced into small pieces and homogenized with 9 vol 0.25 M sucrose solution in a Potter-Elvehjem type Teflon - glass homogenizer. Subsequent fractionation procedures were carried out essentially according to the method of Hogeboom.[12] Preparation of mitochondrial extracts were as follows ; the mitochondrial fraction suspended in 0.25 M sucrose solution was subjected to freeze-thawing and disrupted with ultrasonication for 4 min. The homogenate was centrifuged at 15,000 ×g for 30 min and the precipitate discarded.

Determination of transamination activity : Transamination between either glutamine, glutamate, or albizziin and glyoxylate was in principle determined as described for the method by Cooper and Meister.[13] In this assay medium, 50 mM borate buffer, pH 8.5 was substituted for 50 mM Tris/HCl buffer, pH 8.4. The assay mixture contained, unless otherwise specified, either 20 mM glutamine, 20 mM glutamate, or 20 mM albiz-

ziin, 20 mM glyoxylate, 50 mM borate buffer (pH 8.5), 1 mM EDTA, and either the liver extract, mitochondrial extract, or post-mitochondrial fraction. Incubation was done at 37℃ for 1 hr. The reaction was terminated by adding 0.5 vol 20% trichloroacetic acid or 1 vol 6% sulfosalicylic acid and the insoluble precipitates were removed by centrifugation. The formation of glycine was determined with a Hitachi amino acid analyzer. The protein concentration was determined according to Lowry *et al.*[14] with bovine serum albumin as a standard. Transamination activity was estimated from the glycine formation rate.

Results

Effect of rhatannin on hepatic glutamine transamination in different metabolic situations

In order to clarify the mechanism by which rhatannin causes liver glutamine transamination to enhance, the effect of rhatannin on the transamination was determined with the use of rats under the following dietary conditions : carbohydrate feeding, protein feeding, and fasting ; where the plasma glucagon levels are changed.

Figure 1 shows the glycine formation rates in three different metabolic situations and the influences by rhatannin on their rates. In each control animal, the glycine formation rates were 0.71 ± 0.07 μmol/mg protein/hr, 0.63 ± 0.03 μmol/mg protein/hr, and 0.75 ± 0.03 μmol/mg protein/hr, in rats under carbohydrate feeding, protein feeding, and fasting, respectively.

Rhatannin caused the glycine formation rate to enhance in both rats under protein feeding and under fasting, the increment being 7% and 8%, respectively, as compared with individual control values. In contrast, rhatannin failed to increase the formation rate in rat under carbohydrate feeding. These results suggested that the expression of the enhancing action of rhatannin on the transamination might be closely associated with the hormonal environment since its positive effect was observed only in carbohydrate-restricted rats.

Inhibitory effect of cortisone on the increase in glutamine transamination by rhatannin

It was interesting to determine whether glucocorticoid participated in the promotion of hepatic glutamine transamination. Rats were treated

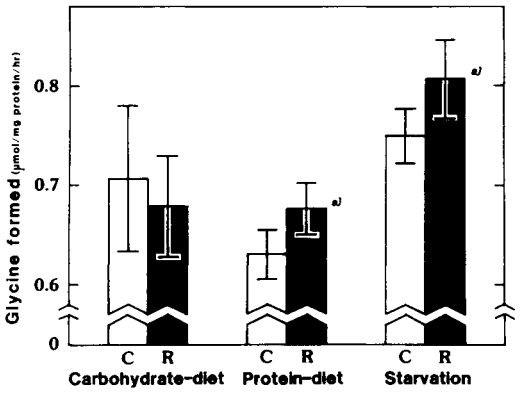

Fig. 1 Effect of dietary conditions on glycine formation from glyoxylate and glutamine in rat liver homogenate, and the influence of rhatannin.

Reaction mixture initially contained liver extract, 50 mM borate buffer (pH 8.5), 1 mM EDTA, 20 mM glyoxylate, and 20 mM glutamine. Experimental conditions were described in Materials and Methods. Data are given as means±S.D. of 5 rats. a) $p<0.05$, Student's t test. C, control rats ; □ : R, rhatannin-treated rats ; ■.

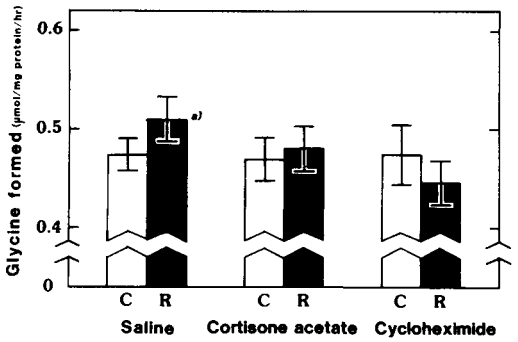

Fig. 2 Effect of cortisone acetate or cycloheximide on the rhatannin-stimulated increase in glycine formation.

Rats fasted for 24 hr were administered cortisone acetate, cycloheximide, or saline 30 min prior to rhatannin treatment, and the glycine formation rate was estimated 4 hr after rhatannin treatment. Data are expressed as means ±S.D. of 5 rats. a)$p<0.05$, Student's t test. C, control rats ; □ : R, rhatannin-treated rats ; ■.

with cortisone acetate 30 min prior to rhatannin treatment. As illustrated in Fig. 2, administration of the glucocorticoid prevented the increase in the glutamine transamination induced by rhatannin. Cortisone alone had no significant effect on the baseline level of the transamination. The result with cycloheximide is consistent with the previous observation[5] that the elevated level of glycine formation induced by rhatannin was completely abolished by the reagent.

Comparison of effects of rhatannin, glucagon, and DBcAMP on hepatic glutamine transamination

In order to clarify the underlying mechanism by which the hepatic glutamine transamination was caused to enhance in rhatannin-treated rat, effects of glucagon and DBcAMP on the transamination between either glutamine, glutamate, or albizziin and glyoxylate were tested under the present experimental condition. In addition, the effects of rhatannin on these transaminations were compared with those of glucagon and DBcAMP.

Figure 3 shows the production rates of glycine from glyoxylate with each amino-donor and the effect of rhatannin, glucagon, and DBcAMP on their rates. The glycine formation rates in control animals were 0.58 μmol/mg protein/hr, 0.35 μmol/mg protein/hr, and 0.18 μmol/mg protein/hr, with glutamine, glutamate, and albizziin as amino-donors, respectively. Rhatannin, glucagon, and DBcAMP had a stimulatory effect on the transamination between glutamine and glyoxylate. The increments were 11%, 16%, and 11%, respectively, as compared with individual control values. Although glutamate and albizziin could partially substitute for glutamine, transaminations from these amino-donors failed to enhance in any treatment. These results indicated that the glutamine-glyoxylate transamination was responsible for the increase in the glycine formation rates induced by rhatannin, glucagon, and DBcAMP.

Subcellular distribution of the enhanced glutamine transamination reaction induced by rhatannin and glucagon

The subcellular distribution of the enhanced glutamine transamination was determined to provide further evidence for similarity of the observed effects in both rhatannin-treated and glucagon-treated animals. The transamination

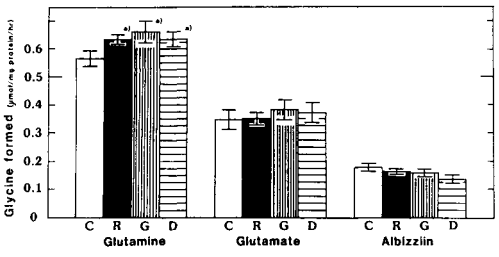

Fig. 3 Effects of glutamine, glutamate, and albizziin as amino-donors to glyoxylate on glycine formation in rat liver homogenate, and the influences of rhatannin, glucagon, and DBcAMP.

The reaction mixture initially contained liver extract, 50 mM borate buffer (pH 8.5), 1 mM EDTA, and either 20 mM glutamine, 20 mM glutamate, or 20 mM albizziin, and 20 mM glyoxylate. Rats fasted for 24 hr were administered rhatannin, glucagon, DBcAMP, or saline, and the animals were sacrificed 4 hr after the treatment. Data are expressed as means±S.D. of 5 rats. a) $p<0.05$, Student's t test. C, control rats ; □ : R, rhatannin-treated rats ; ■ : G, glucagon-treated rats ; ▤ : D, DBcAMP-treated rats ; ▥.

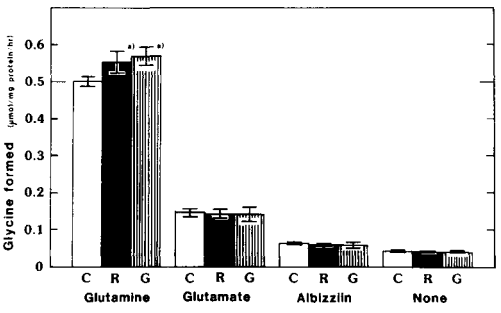

Fig. 4 Effect of glutamine, glutamate, albizzin as amino-donors to glyoxylate on glycine formation in rat liver mitochondrial fraction, and the influences of rhatannin and glucagon.

The reaction mixture initially contained rat liver mitochondrial extract, 50 mM borate buffer (pH 8.5), 1 mM EDTA, 20 mM glyoxylate, and either, 20 mM glutamine, 20 mM glutamate, or 20 mM albizziin. Rhatannin and glucagon were injected intraperitoneally to rats fasted for 24 hr and the animals were sacrificed 4 hr after the treatment. Data are expressed as means±S.D. of 5 rats. a) $p<0.05$, Student's t test. C, control rats ; □ : R, rhatannin-treated rats ; ■ : G, glucagon-treated rats ; ▥.

rates were estimated with the use of mitochondrial and post-mitochondrial fractions. Figure 4 illustrates the glycine formation rate with the mitochondrial fraction and the influences of rhatannin and glucagon. The glycine formation rates in control animals were 0.50 μmol/mg protein/hr, 0.15 μmol/mg protein/hr, 0.06 μmol/mg protein/hr, and 0.04 μmol/mg protein/hr, with glutamine, glutamate, albizziin, and endogeneous substrate as amino-donors, respectively. The transamination between glutamine and glyoxylate was enhanced in rhatannin-treated rats. Glucagon shared the enhancing action on the glutamine-glyoxylate transamination. The increments induced by rhatannin and glucagon were 10% and 14%, respectively, as compared with the control value. Other glycine formations were not affected by rhatannin and glucagon. In contrast, both rhatannin and glucagon failed to exhibit a stimulatory effect on any glutamine transamination in postmitochondrial fraction, as illustrated in Fig. 5. These results showed that the enhanced transamination activity existed in hepatic mitochondrial fraction in both rhatannin-treated and glucagon-treated rats. Further, the inhibitory effect of cortisone on the enhanced glutamine transamination in mitochondrial fraction induced by rhatannin and glucagon was tested. Table I shows that the administration of cortisone 30 min prior to rhatannin and glucagon treatment completely prevented their enhancing action to the transamination in mitochondrial fraction.

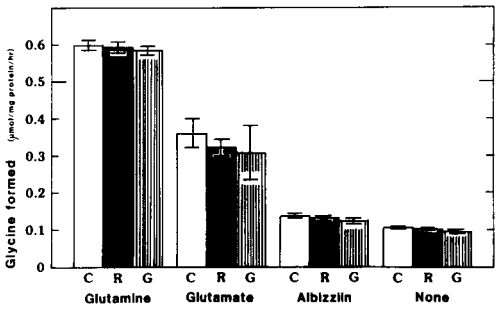

Fig. 5 Effect of glutamine, glutamate, albizziin as amino-donors to glyoxylate on glycine formation in rat liver post-mitochondrial fraction and the absence of rhatannin- or glucagon-stimulated increase in glutamine-glyoxylate transamination.
The experimental condition was described in Fig. 4, except that the rat liver post-mitochondrial fraction was substituted for the liver mitochondrial extract in the reaction mixture. C, control rats ; □ : R, rhatannin-treated rats ; ■ : G, glucagon-treated rats ; ▨.

Discussion

It is well known that glucagon served as a potent inducer to a number of amino acid metabolizing enzymes in glutamine transamination. For example, Noguchi *et al.*[7-9] showed that rat tissue possesses two enzymes capable of catalyzing transamination between glutamine and glyoxylate : these two enzymes were designated I and II. Enzyme I, which was shown to be present only in the liver, was inducible by glucagon and

Table I Effects of cortisone acetate on rhatannin- and glucagon-induced increase to the glycine formation in rat liver mitochondrial fraction and post-mitochondrial fraction.

	No. of rats	Glycine formed μmol/mg protein/hr	
		Mitochondrial fr.	Post-mitochondrial fr.
Control	5	0.64±0.02 (100)	0.57±0.02 (100)
Rhatannin	5	0.62±0.06 (97)	0.58±0.03 (102)
Glucagon	5	0.61±0.02 (95)	0.60±0.02 (105)

Rats were treated with cortisone acetate 30 min prior to rhatannin and glucagon treatment and were sacrificed 4 hr after the treatment. Data are expressed as means±S.D.

was identical with serine-pyruvate transaminase. Further, Fukushima *et al*.[15] demonstrated that the accumulation of serine-pyruvate transaminase in rat liver mitochondria by glucagon treatment was due to the rise in the rate of enzyme synthesis. Enzymatically, the increase in this activity was prevented or partially prevented by treatment with cycloheximide or cortisone, respectively.[14] The other enzyme, enzyme II, was identical to glutamine transaminase which was non-inducible by glucagon.[16] This glutamine transaminase is separated two types (L and K types),[10,11] which were distinguished by their relative activities toward albizziin as amino-donor. Albizziin in place of glutamine is able to be utilized effectively by glutamine transaminase L, whereas albizziin is a poor substrate of glutamine transaminase K.

The present data showed that rhatannin caused the glycine formation from glyoxylate to increase with glutamine as amino-donor but not with glutamate and albizziin. This increased activity in glutamine transamination induced by rhatannin existed in liver mitochondrial fraction and was prevented by cortisone treatment. In this connection, glucagon might serve the same action to the metabolic responses as that of rhatannin. Therefore, the present result suggests that the mechanism by which rhatannin caused the transamination to enhance might be mediated through its action on the glucagon regulatory pathway. It seems to be likely that increased activity of serine-pyruvate transaminase might have participated in the increase in the transamination activity. However, it is obscure that any kind of enzymic activity was contributed to the enhancement in the glutamine-glyoxylate transamination.

Unger[17] has suggested that insulin-to-glucagon molar (I/G) ratio is more important in determining the metabolic responses of the target organs than the absolute concentration of either and the I/G ratio is influenced by antecedent diet and energy availability. The low I/G ratio exhibits a net glucagon-like effect, while insulin-like activity is a dominant influence in high I/G ratio. Parrilla *et al*.[18] demonstrated with the use of the isolated perfused rat liver that glucagon and insulin have antagonistic metabolic effects and the I/G ratio is the important signal regulating the rate of gluconeogenesis, ketogenesis, ureogenesis, glycogenolysis or lactate production.

The present experiment indicates that rhatannin caused the glutamine transamination to increase under the condition of carbohydrate-deficiency, regardless of whether the animal was fed or fasted, for rhatannin failed to exert a positive effect when the need for gluconeogenesis was abolished by carbohydrate abundance. Therefore, the present data suggests that the increased transamination induced by rhatannin might be closely associated with the promotion of gluconeogenesis. In addition, glucagon shared the increase of the transamination under the present experimental condition. It seems likely that the involvement of glucagon in gluconeogenesis might reflect the increase on the glutamine transamination. If this is the case, a question arises as to why rhatannin exerted the glucagon-like effect. This is possible when the stimulatory effect of rhatannin to metabolic response was mediated by the glucagon-like effect as the result of low I/G ratio. As discussed above, the changes in the glutamine transamination may be interpreted in terms of I/G ratio. Consequently, three possibilities seem reasonable. The first is that rhatannin produced either an increased response to glucagon activity or a decreased response to insulin suppression. Next rhatannin affected the absolute concentrations of either or both hormones in blood. Finally, rhatannin itself affected another pathway.

Using isolated rat hepatocytes, the expression of enhancing action of rhatannin on gluconeogenesis required the presence of glucagon, although rhatannin itself failed to cause this process to promote.[6] These results suggest that rhatannin exerted synergistical action for glucagon activity on the gluconeogenic pathway. It seems to be likely *in vivo* that rhatannin might produce an increase response to the metabolic effect of glucagon. Therefore, the present experiment strongly suggests that the expression of the increase of glutamine transamination caused by rhatannin might be mediated by a net glucagon-

like effect since rhatannin could exert this process to enhance only in the carbohydrate-restricted animal situation which is associated with a low I/G ratio. However, the present data cannot exclude another possibility, since levels of the hormones have not yet been determined. This experiment does not offer any conclusions about the site of the regulatory mechanism of glucagon influenced by rhatannin.

和文抄録

Rhatanninのグルタミンアミノ基転移反応に及ぼす影響を蛋白食あるいは炭水化物食飼育ラット並びに絶食ラットを用い検討した。アミノ基転移活性はグルタミンとグリオキシル酸を基質に産生されるグリシン量を測定し算出した。その結果，rhatanninは，蛋白食飼育ラット及び絶食ラットにおいてのみその活性を増加させ，炭水化物食飼育ラットにおいては何ら影響を示さなかった。この活性増加はグルカゴン並びにジブチリルサイクリックAMP投与によっても認められた。Rhatannin投与あるいはグルカゴン投与により増加するアミノ基転移活性は，アミノ基供給体として検討したグルタミン，グルタミン酸並びにアルビジンのうちグルタミンにのみ特異的であった。この増加する活性は肝ミトコンドリア画分に存在した。また，コルチゾンの前処置により，rhatannin並びにグルカゴンの示す作用は消失した。以上今回の実験結果は，グルタミンのアミノ基転移反応の亢進によりグリシン産生の亢進が糖新生の亢進と関連している可能性を示唆するとともに，rhatanninの示すグルタミンアミノ基転移反応の亢進作用はグルカゴンの作用経路を介して発現した可能性を示唆した。

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