Role of *N*-Acetylglutamate Turnover in Urea Synthesis by Rats Treated with the Thyroid Hormone

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We determined whether the synthesis and degradation of *N*-acetylglutamate would regulate urea synthesis when the thyroid status was manipulated. Experiments were done on three groups of rats, each being given 6-propyl-2-thiouracil (PTU, a thyroid inhibitor) without a triiodothyronine (T₃) treatment, treated with PTU + T₃, or receiving neither PTU nor T₃ (control). The plasma concentration and urinary excretion of urea, the liver concentration of *N*-acetylglutamate, and the liver *N*-acetylglutamate synthesis in rats given PTU alone were each significantly higher than in the control rats. Compared with the control rats, the liver *N*-acetylglutamate degradation was significantly lower in those rats given PTU without the T₃ treatment. Treatment of the PTU-treated rats with T₃ reversed the effects of PTU to the values of the control rats. *N*-Acetylglutamate synthesis in the liver was closely correlated with the excretion of urea, and inverse correlation between the liver *N*-acetylglutamate degradation and urea excretion was found. These results suggest that the greater synthesis and lower degradation of *N*-acetylglutamate in the hypothyroid (PTU alone) rats would be likely to increase the hepatic concentration of this compound and stimulate urea synthesis.

Key words: triiodothyronine; urea synthesis; *N*-acetylglutamate synthesis; *N*-acetylglutamate degradation

Shimke^{1,2)} has suggested that the concentrations of urea-cycle intermediates were unchanged under conditions affecting the rate of urea excretion (e.g., ingestion of a high-protein diet) and concluded that the activities of various urea-cycle enzymes were regulatory factors of urea synthesis. However, we^{3,4)} have previously reported that treating with 6-propyl-2-thiouracil (PTU, a thyroid inhibitor⁵⁾) elevated urea excretion while it reduced the activity of urea-cycle enzymes relative to control animals, and that the thyroid hormone reversed the effects of PTU.

Urea formation has been shown to be stimulated by adding *N*-acetylglutamate during liver perfusion when substrates for urea production were present in excess.^{6,7)} *N*-Acetylglutamate, an essential activator of carbamylphosphate synthetase (EC 6.3.4.16), plays a critical role

in the regulation of urea synthesis in mammalian liver.^{8,9)} Shigesada and Tatibana¹⁰⁾ have demonstrated that *N*-acetylglutamate synthetase (EC 2.3.1.1) catalyzed the acetylation of glutamate in liver. This enzyme is specifically activated by arginine.^{11,12)} On the other hands, Meijer *et al.*^{13,14)} have suggested that there could be an alteration in the enzyme system catabolizing *N*-acetylglutamate in hepatic cytosol or in the system transporting this compound out of the mitochondria under physiological conditions.

The purpose of this present study was to discover the mechanism by which the thyroid hormone reduces urea synthesis. In our previous report,^{15–17)} positive correlations between the liver concentration of *N*-acetylglutamate and urea excretion, and between the plasma concentration of arginine and the liver concentration of *N*-acetylglutamate were found when the thyroid status was manipulated. Two questions were considered in the present study: 1) whether the thyroid hormone might control *N*-acetylglutamate synthesis in the liver and 2) whether a higher degradation of *N*-acetylglutamate in rats treated with PTU and the thyroid hormone might result in a lower *N*-acetylglutamate concentration and less urea synthesis than those animals treated with PTU alone. We therefore examined *in vivo* the hepatic synthesis and degradation of *N*-acetylglutamate by rats treated with the thyroid hormone.

Materials and Methods

Chemicals. 6-Propyl-2-thiouracil, 3,5,3'-triiodothyronine, and *N*-acetylglutamic acid were each purchased from Sigma Chemical (St. Louis, MO, U.S.A.). NaH¹⁴CO₃ (1.85 GBq/mmol) and U-¹⁴C-glutamic acid (9.25 GBq/mmol) were obtained from Amersham (Tokyo, Japan). All other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Animals and diets. Young male Wistar rats*¹ (70–80 g) were maintained at 24°C with a 12 h light: dark cycle. The rats were transferred to the experimental diet or the basal diet after being fed with a commercial nonpurified diet*² for 4 d. The experimental diet contained 0.01% PTU added to the basal diet (Table I). All rats were in-

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Abbreviations: PTU, 6-propyl-2-thiouracil; T₃, triiodothyronine.

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*² MF; Oriental Yeast Co., Tokyo, Japan.

Table I. Composition of the Basal Diet

Ingredient	Amount
	g/100 g of diet
Casein ¹	20.0
L-Cys	0.3
Corn starch ¹	43.3
Sucrose ¹	21.7
Corn oil ²	5.0
Cellulose ¹	5.0
AIN-93G mineral mix ³	3.5
AIN-93VX vitamin mix ³	1.0
Choline chloide	0.2

¹ Supplied by Oriental Yeast Co., Tokyo, Japan.

² Supplied by Yoneyama Reagent Industries, Osaka, Japan.

³ Supplied by Nihon Nosan, Yokohama, Japan.²⁸⁾

dividually housed and provided free access to water. The food intake of each group was adjusted to the mean intake by the rats given only PTU. At the beginning of the experimental period, the body weight of each animal ranged from 90 to 100 g.

The approval of Aichi University of Education Animal Care and Use Committee was given for our animal experiments.

Experimental procedures. Three experiments were done, with 18 rats (Experiments 1 and 2) or 54 rats (Experiment 3) being divided randomly into three groups. After the experimental period, the rats were decapitated under anesthesia with ethyl-ether and the plasma was collected in glass tubes and stored at -20°C . The livers were quickly removed, weighed, and used.

Experiment 1. The effects of the thyroid hormone on the urinary excretion of urea, the plasma concentration of urea and the hepatic concentration of *N*-acetylglutamate were investigated. The animals were fed on the basal or experimental diet for 14 d. Two groups were fed on the experimental diet and subcutaneously injected with either triiodothyronine [0.1 mg/(100 g of body wt·d)] or 0.9% NaCl for the last 5 d of the 14-d experimental period (9:00 a.m.). The injected volume was 0.1 ml/100 g of body wt. The third group was fed the basal diet and injected with saline. On day 12–13, urine was collected for 24 h (10:00 a.m.–10:00 a.m.), filtered, and used for analyzing urea.

Experiments 2 and 3. The effects of the thyroid hormone on *N*-acetylglutamate synthesis (Experiment 2) and *N*-acetylglutamate degradation (Experiment 3) in the liver were studied. The same experimental procedure as that in Experiment 1 was used.

Analytical procedures. The plasma and urinary concentrations of urea were measured by the method of Archibald¹⁸⁾ and the 3,5,3'-triiodo-L-thyronine concentration in the plasma was measured by RIA.¹⁹⁾ The *N*-acetylglutamate concentration was measured by the method described in our previous report⁴⁾ and the hepat-

Table II. Time-dependent Changes in the Specific Radioactivity of Free Glutamate in the Liver of Rats Fed on the 6-Propyl-2-thiouracil (PTU)-supplemented Diets with or without the Thyroid Hormone Treatment, and of the Control Rats¹

Time after injection	Control	PTU	PTU+T ₃	Pooled SEM
(min)	Bq/ μmol			
5	2700	2610	2850	140
15	2450	2380	2590	130
30	430	400	460	40
60	200	190	220	20
120	200	180	210	20

¹ Each values is the mean and pooled SEM of four rats. Values within each time period were not significantly different ($p>0.05$). T₃ is triiodothyronine.

ic synthesis and degradation of *N*-acetylglutamate were determined according to the methods of Shigesada *et al.*¹¹⁾ and Morita *et al.*²⁰⁾ Radioactive U-¹⁴C-glutamate was combined with unlabelled glutamate acid to yield a dose of 1.11 MBq and a concentration of 5 $\mu\text{mol}/\text{ml}$ of saline. The rats were each given an intraperitoneal injection at a dose of 1 ml/100 g of body wt. After 15 min, the rats were quickly decapitated in Experiment 2. In Experiment 3, the rats were decapitated 30, 60 and 120 min after the injection. The measurement of *N*-acetylglutamate synthesis and degradation involved isolating *N*-acetylglutamate and then radioactivity counting.^{*3}

In a preliminary experiment, we determined the hepatic specific radioactivity of glutamate 5, 15, 30, 60 and 120 min after the injection of radioactive glutamate under these experimental conditions by the method of Morita *et al.*,²⁰⁾ and checked whether the change in radioactivity of *N*-acetylglutamate in the liver would be affected by a precursor pool of this compound. The specific radioactivity of glutamate in the liver was maximal 5–15 min after the injection, and decreased to very low values by 30, 60 and 120 min (Table II). These values in the three groups were the same, indicating that the prolonged retention of radioactivity in *N*-acetylglutamate was not due to continued entry of the label into this compound but instead to an increase in the half-life of the compound in Experiment 3.

Statistical analyses. The mean and pooled SEM are reported. Duncan's multiple-range test was used to compare means after one-way ANOVA.^{21,22)} A linear regression analysis was used to assess the liver degradation of *N*-acetylglutamate,²²⁾ differences being considered significant at $p<0.05$.

Results

Effect of the thyroid hormone treatment on the liver concentration of N-acetylglutamate (Experiment 1)

The effects of the thyroid hormone on the urea concentration in plasma and its output in urine, and on the liver concentration of *N*-acetylglutamate are presented in Table III. The PTU+T₃ treatment caused a significantly lower body weight gain measured over the 5-d T₃ or saline treatment period than in the rats treated

*3 LS 5000TD; Beckman Japan, Tokyo, Japan.

Table III. Effects of the Thyroid Hormone on the Urinary and Plasma Urea Concentrations and Liver Concentration of *N*-Acetylglutamate in Rats Fed on the 6-Propyl-2-thiouracil (PTU)-supplemented Diets (Experiment 1)¹

	Control	PTU	PTU + T ₃	Pooled SEM
Body weight gain, ² g/5 d	16.9 ^a	12.3 ^b	5.9 ^c	0.7
Liver weight, g/100 g of body wt.	4.40	4.31	4.24	0.11
Urinary urea, mmol/d	5.50 ^b	7.21 ^a	4.75 ^c	0.19
Plasma urea, mmol/liter	5.00 ^b	7.91 ^a	3.74 ^c	0.20
Plasma T ₃ , nmol/liter	1.70 ^b	0.65 ^c	9.74 ^a	0.30
Food intake, ³ g/5 d	68.3	68.3	68.3	
Liver <i>N</i> -acetylglutamate, nmol/g of liver	19.5 ^b	25.5 ^a	18.4 ^b	0.6

¹ Each values is the mean and pooled SEM of six rats. Means with different superscript letters are significantly different ($p < 0.05$). T₃ is triiodothyronine.² Body weight gain only during the 5 d of the T₃ or saline treatment is presented.³ Food intake of the group treated with PTU + T₃ and of the control group was adjusted to the mean intake of the group given PTU alone.**Table IV.** Effects of the Thyroid Hormone on the Urinary and Plasma Concentrations of Urea and Liver Synthesis of *N*-Acetylglutamate in Rats Fed on the 6-Propyl-2-thiouracil (PTU)-supplemented Diets (Experiment 2)¹

	Control	PTU	PTU + T ₃	Pooled SEM
Body weight gain, ² g/5 d	17.6 ^a	13.3 ^b	6.2 ^c	0.8
Liver weight, g/100 g of body wt.	4.39	4.28	4.21	0.13
Urinary urea, mmol/d	5.20 ^b	6.82 ^a	4.59 ^c	0.16
Plasma urea, mmol/liter	5.21 ^b	7.14 ^a	3.40 ^c	0.19
Plasma T ₃ , nmol/liter	1.72 ^b	0.61 ^c	9.52 ^a	0.28
Food intake, ³ g/5 d	68.0	68.0	68.0	
Liver <i>N</i> -acetylglutamate synthesis, Bq/g of liver	19.2 ^b	38.8 ^a	17.5 ^b	1.5

¹ Each values is the mean and pooled SEM of six rats. Means with different superscript letters are significantly different ($p < 0.05$). T₃ is triiodothyronine.² Body weight gain only during the 5 d of the T₃ or saline treatment is presented.³ Food intake of the group treated with PTU + T₃ and of the control group was adjusted to the mean intake of the group given PTU alone. **Fig.** Changes in the Total Radioactivity of Liver *N*-Acetylglutamate in Rats Given 6-Propyl-2-thiouracil (PTU) without the Triiodothyronine (T₃) Treatment, Those Treated with PTU + T₃ and Those Receiving neither PTU nor T₃ (Control).Each values are expressed as the mean $\pm$ SEM, $n=6$ (Experiment 3).

with PTU alone. The group given PTU alone grew less over this period than the control group. The liver weight was not significantly different among the three groups when expressed relative to the body weight. Compared with the control rats, the plasma concentration of T₃ was significantly lower in those rats given PTU without the T₃ treatment. Urinary excretion and the plasma concentration of urea, and the hepatic concentration of *N*-acetylglutamate in those rats treated with PTU alone were significantly greater than in the control rats. Treatment of the PTU-treated rats with T₃ tended to reverse these effects of PTU to approach the values of the control group. The liver concentration of *N*-acetylgluta-

mate was correlated to the excretion of urea ($r=0.901$, $p < 0.001$).

Effect of the thyroid hormone treatment on the liver synthesis of N-acetylglutamate (Experiment 2)

Table IV shows the effects of the thyroid hormone on the plasma concentration of urea, the urea excreted in urine and *N*-acetylglutamate synthesis in the liver. As in Experiment 1, the group receiving PTU + T₃ grew less over the 5-d T₃ treatment than the group given PTU alone. The urinary excretion and plasma concentration of urea, and the hepatic synthesis of *N*-acetylglutamate in those rats treated with PTU alone were significantly higher than the levels in the control rats. The PTU + T₃ treatment reversed the effects of PTU as described in Experiment 1.

Effect of the thyroid hormone treatment on the liver degradation of N-acetylglutamate (Experiment 3)

Time-course plots for the radioactivity of hepatic *N*-acetylglutamate in the rats treated with the thyroid hormone are presented in the Figure. Table V shows the degradation rate constant and half-life for *N*-acetylglutamate in the liver, and the urea excretion in the urine. As in Experiments 1 and 2, urinary excretion of urea was significantly greater by those rats given PTU alone than by those rats given PTU + T₃ or by the control rats. The radioactivity in hepatic *N*-acetylglutamate in the PTU-treated rats without the T₃ treatment decreased more slowly with a half-life of 63 min than that in the control rats. Compared with the control rats, the degradation rate constant of hepatic *N*-acetylglutamate was sig-

Table V. Effects of the Thyroid Hormone on the Urinary Excretion of Urea, and Hepatic Degradation Rate Constant and Half-Life of *N*-Acetylglutamate in Rats Fed on the 6-Propyl-2-thiouracil (PTU)-supplemented Diets (Experiment 3)

	Urinary urea ¹	Degradation rate constant (kd) ²	Half-life
	mmol/d	h ⁻¹	min
Control	5.10 ^b	0.924 ± 0.023 ^a	45
PTU	6.71 ^a	0.673 ± 0.020 ^b	62
PTU + T ₃	4.45 ^c	0.990 ± 0.027 ^a	42
Pooled SEM	0.18		

¹ Each values is the mean and pooled SEM of six rats. The urine from those rats killed 30 min after injecting the radioisotope was collected in this experiment for 24 h on day 12–13. Means with different superscript letters are significantly different ($p < 0.05$). T₃ is triiodothyronine.

² A linear regression analysis was used to determine the degradation rate constant of *N*-acetylglutamate in the liver. Each value is the mean ± SEM.

nificantly lower in those rats treated with PTU alone. The PTU + T₃ treatment reversed the effects of PTU to approach the values of the control group. In the control and PTU + T₃-treated rats, the half-lives in hepatic *N*-acetylglutamate were 45 and 42 min, respectively.

Discussion

N-Acetylglutamate is a physiological activator of carbamylphosphate synthetase.^{8,23} Variation in the *N*-acetylglutamate concentration has correlated well with urea synthesis in intact animals^{10,11,15–17} and in isolated hepatocytes.^{10,24} Many investigators have indicated that a change in *N*-acetylglutamate synthesis was involved in regulating the liver concentration of *N*-acetylglutamate.^{11,23} However, little documentation on the effect of the thyroid hormone on *N*-acetylglutamate synthesis *in vivo* is available. Therefore, we assumed that the thyroid hormone would regulate the hepatic synthesis of *N*-acetylglutamate. Treatment with PTU alone increased the concentration and synthesis of *N*-acetylglutamate in the liver, and T₃ reversed the effects of PTU (Table IV). In the present study, the greater synthesis of liver *N*-acetylglutamate in the PTU-treated group enhanced the *N*-acetylglutamate concentration in this group. The change in *N*-acetylglutamate synthesis may have controlled the *N*-acetylglutamate concentration and been one of the factors affecting urea synthesis in this study (Tables III and IV). *N*-Acetylglutamate synthetase has been shown to catalyze the acetylCoA-dependent acetylation of glutamate in liver mitochondria.¹⁰ This enzyme is activated by arginine.^{11,12} Shigesada *et al.*¹¹ have demonstrated that an arginine treatment stimulated the hepatic synthesis of *N*-acetylglutamate *in vivo* and in isolated hepatocytes. In our previous study,¹⁵ we suggested that the positive correlation between the plasma concentration of arginine and the liver concentration of *N*-acetylglutamate was induced when the thyroid status was manipulated, and that the activity of *N*-acetylglutamate synthetase and the concentration of glutamate in the liver was not related to the concentration of *N*-acetylglutamate. Further studies should seek to determine whether the plasma arginine concentration may regulate hepatic *N*-acetylglutamate synthesis when

the thyroid status is changed.

N-Acetylglutamate is transported from mitochondria to cytosol in the liver, and then degraded. The activity of the catabolizing enzyme was detected only in the cytosol.^{25,26} The hepatic concentration and half-life of *N*-acetylglutamate increased markedly after protein ingestion.²⁰ Thus, Morita *et al.*²⁰ have reported that the *in vivo* degradation contributed to the concentration of *N*-acetylglutamate when protein ingestion was changed. Therefore, we assume that the thyroid hormone also regulated *N*-acetylglutamate degradation. In this study, not only greater synthesis but also lower degradation of *N*-acetylglutamate in the liver may have induced a greater concentration of this compound in the PTU-treated group without the T₃ treatment. The regulation of urea synthesis may be partly mediated by an alteration of *N*-acetylglutamate degradation when the thyroid status is manipulated. The mechanism by which the thyroid hormone affects the hepatic degradation process of *N*-acetylglutamate remains to be determined. Since the hepatic cytosol fraction has a high level of *N*-acetylglutamate catabolizing activity,^{25,26} an indication of limiting *N*-acetylglutamate degradation by its transport out of the mitochondria has been reported by many investigators.^{13,14,20,27} The rate of *N*-acetylglutamate efflux through the mitochondrial membrane and its degradation have been found to be lower in rats fed on a high-protein diet than in those fed on a protein-free diet or fasted rats.²⁰ This is another possibility to consider in further examinations of the mechanism by which the thyroid hormone alters urea synthesis.

The results suggest that the greater synthesis and lower degradation of *N*-acetylglutamate in the hypothyroid (PTU treatment alone) are likely to increase the hepatic concentration of this compound and stimulate urea synthesis.

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References

- 1) R. T. Schimke, *J. Biol. Chem.*, **237**, 459–468 (1962).
- 2) R. T. Schimke, *J. Biol. Chem.*, **238**, 1012–1018 (1963).
- 3) K. Hayase, G. Yonekawa, H. Yokogoshi, and A. Yoshida, *J. Nutr.*, **121**, 970–978 (1991).
- 4) K. Hayase, G. Yonekawa, and A. Yoshida, *J. Nutr.*, **122**, 1143–1148 (1992).
- 5) S. Shimoda, *Acta Endocrinol.*, **46**, 653–664 (1964).
- 6) T. Saheki and N. Katunuma, *J. Biochem.*, **77**, 659–669 (1975).
- 7) T. Saheki, M. Tsuda, T. Tanaka, and N. Katunuma, *J. Biochem.*, **77**, 671–678 (1975).
- 8) K. Shigesada and T. Tatibana, *J. Biol. Chem.*, **246**, 5588–5595 (1971).
- 9) K. Shigesada and T. Tatibana, *Biochem. Biophys. Res. Commun.*, **44**, 1117–1124 (1971).
- 10) K. Shigesada and T. Tatibana, *Eur. J. Biochem.*, **84**, 285–291 (1978).
- 11) K. Shigesada, K. Aoyagi, and T. Tatibana, *Eur. J. Biochem.*, **85**, 385–391 (1978).
- 12) E. S. Kamemoto and D. E. Atkinson, *Arch. Biochem. Biophys.*, **243**, 100–107 (1985).
- 13) A. J. Meijer and G. M. Van Woerkom, *Biochim. Biophys. Acta*, **721**, 240–246 (1982).
- 14) A. J. Meijer, G. M. Van Woerkom, R. J. A. Wanders, and C.

- Lof, *Eur. J. Biochem.*, **124**, 325–330 (1982).
- 15) K. Hayase, G. Yonekawa, and A. Yoshida, *J. Nutr.*, **123**, 253–258 (1993).
- 16) K. Hayase and A. Yoshida, *Biosci. Biotech. Biochem.*, **57**, 1722–1725 (1993).
- 17) K. Hayase and A. Yoshida, *Biosci. Biotech. Biochem.*, **59**, 801–804 (1995).
- 18) R. M. Archibald, *J. Biol. Chem.*, **157**, 507–518 (1945).
- 19) J. G. Brown and D. J. Millward, *Biochim. Biophys. Acta*, **757**, 182–190 (1983).
- 20) T. Morita, M. Mori, and M. Tatibana, *J. Biochem.*, **91**, 563–569 (1982).
- 21) D. B. Duncan, *Biometrics*, **11**, 1–42 (1955).
- 22) G. W. Snedecor and W. G. Cochran, in “Statistical Methods,” 6th ed., Iowa State University Press, Ames, IA, 1967, pp. 135–171.
- 23) P. M. Stewart and M. Walser, *J. Biol. Chem.*, **255**, 5270–5280 (1980).
- 24) H. E. S. J. Hensgens, A. J. Vorhoeven, and A. J. Meijer, *Eur. J. Biochem.*, **107**, 197–205 (1980).
- 25) J. D. McGivan, N. M. Bradford, and J. Mendes-Mourao, *Biochem. J.*, **154**, 415–421 (1976).
- 26) A. Reglero, J. Rivas, J. Mendelson, R. Wallace, and S. Grisolia, *FEBS Lett.*, **81**, 13–17 (1977).
- 27) A. J. Meijer, W. H. Lamers, and W. A. F. M. Chamuleau, *Physiol. Rev.*, **70**, 701–748 (1990).
- 28) American Institute of Nutrition, *J. Nutr.*, **123**, 1939–1951 (1993).