Role of Ornithine Transport into Mitochondria in Urea Synthesis of Rats Treated with Thyroid Hormone

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Summary The purpose of this study was to find whether or not the ornithine transport into mitochondria regulated urea synthesis when the thyroid status is manipulated. Experiments were done on three groups of rats: given 6-propyl-2-thiouracil (PTU, a thyroid inhibitor) without triiodothyronine (T3) treatment, treated with PTU + T3, or receiving neither PTU nor T3 (control). The urinary excretion of urea, liver concentration of ornithine and ornithine transport into isolated hepatic mitochondria in rats given PTU + T3 were significantly lower than in rats given PTU alone. Ornithine transport was significantly inhibited by the addition of lysine specifically. This response was achieved well within the physiological concentration of lysine. Compared with rats given PTU without T3 treatment, the liver concentration of lysine was significantly higher in rats treated with PTU + T3 and control rats. Ornithine transport into hepatic mitochondria was closely correlated with the excretion of urea. The results suggest that the greater ornithine transport in the hypothyroid (PTU alone) rats is likely to stimulate urea synthesis. A thyroid hormone-induced increase in lysine concentration may be at least partly responsible for the changes in ornithine transport into mitochondria.

Key Words triiodothyronine, urea synthesis, lysine, ornithine transport, thyroid hormone

Schimke (1, 2) 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 in urea synthesis. However, we (3–5) reported that 6-propyl-2-thiouracil (PTU, a thyroid inhibitor) treatment elevated urea excretion while it reduced the activities of urea cycle enzymes relative to control

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Abbreviations: PTU, 6-propyl-2-thiouracil; T3, triiodothyronine.
animals, and that thyroid hormone reversed the effects of PTU.

Urea formation has been shown to be stimulated by an additional supply of ornithine in vivo (6) in perfused liver (7,8) and in isolated hepatocytes (9) when substrates for urea production are present in excess. Thus, the supply of ornithine rather than enzyme activity may be one of the factors regulating urea synthesis.

The purpose of this study was to elucidate the mechanism by which thyroid hormone reduces urea synthesis. In our previous reports (3,4,10), positive correlations between the liver concentration of ornithine and urea excretion were found when the thyroid status was manipulated. However, as ornithine trans-carbamylase (EC 2.1.3.3) is distributed in hepatic mitochondria, ornithine synthesized by arginase (EC 3.5.3.1) must be transported into the mitochondria. Cohen et al. (11) and Zollner (12) suggested that under physiological conditions, the transport of ornithine into the hepatic mitochondria limits the rate of urea production and that lysine inhibits the ornithine transport system. Three questions were considered in this study: 1) whether a thyroid hormone might control ornithine transport into hepatic mitochondria; 2) whether ornithine transport is inhibited within the physiological concentration by lysine treatment specifically; and 3) whether a higher concentration of lysine in rats treated with PTU and thyroid hormone might result in reduced ornithine transport into mitochondria and reduced urea synthesis as compared to rats treated with PTU alone. Therefore, we examined ornithine transport into isolated hepatic mitochondria and the liver concentration of lysine in rats treated with a thyroid hormone. The effects of adding amino acids in vitro on ornithine transport were also investigated.

MATERIALS AND METHODS

**Chemicals.** 6-Propyl-2-thiouracil and 3,5,3'-triiodo-L-thyronine were purchased from Sigma Chemical (St. Louis, MO, USA). [2,3-3H]Ornithine (1.11 TBq/mmol) was purchased from Moravek Biochemicals (Tokyo, Japan). U-14C-Sucrose (17GBq/mmol) was obtained from Amersham (Tokyo, Japan). All other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan).

**Animals and diets.** Young male rats of the Wistar strain (70–80 g, Japan SLC, Hamamatsu, Japan) were maintained at 24°C with a 12 h light:dark cycle. The rats were transferred to the experimental diet or basal diet after eating a commercial nonpurified diet (MF; Oriental Yeast, Tokyo, Japan) for 4 d. The experimental diet contained 0.01% PTU added to the basal diet (Table 1). All rats were individually housed and provided free access to water. Food intake of all groups was adjusted to the mean intake of the rats given PTU only. At the beginning of the experimental period, the body weights of animals ranged from 90 to 100 g.

**Experimental design.** Three experiments were done. Eighteen rats were divided randomly into three groups in all experiments. At the end of the experi-
Table 1. Composition of the basal diet.

| Ingredient                          | Amount (g/100 g diet) |
|-------------------------------------|------------------------|
| Casein\(^1\)                       | 20.0                   |
| L-Cys                               | 0.3                    |
| Corn starch\(^1\)                   | 43.3                   |
| Sucrose\(^1\)                       | 21.7                   |
| Corn oil                            | 5.0                    |
| Cellulose\(^1\)                     | 5.0                    |
| AIN-93G mineral mix\(^2\)           | 3.5                    |
| AIN-93VX vitamin mix\(^2\)          | 1.0                    |
| Choline chloride                    | 0.2                    |

\(^1\) Supplied by Oriental Yeast, Tokyo, Japan.
\(^2\) Supplied by Nihon Nosan, Yokohama, Japan (27).

mental period, rats were decapitated and plasma was collected into glass tubes using heparin and stored at \(-20^\circ C\). Livers were quickly removed, weighed and used immediately.

**Experiment 1.** The effects of thyroid hormones on the urinary excretion of urea, and the hepatic concentrations of ornithine and lysine were investigated. Animals were fed basal or experimental diets for 14 d. Two groups were fed the experimental diet and injected subcutaneously with either triiodothyronine \([0.1 \text{mg/} (100 \text{g body wt\cdot d})]\) or 9 g/L NaCl for the last 5 d of the 14-d experimental period. The injected volume was 0.1 mL/100 g body wt. The third group was fed the basal diet and injected with saline. On days 12–13, urine was collected for 24 h, filtered and used for analysis of urea.

**Experiment 2.** The effects of the addition of all amino acids in vitro on ornithine transport into hepatic isolated mitochondria. Rats were fed the basal diet for 7 d. Ornithine transport into isolated hepatic mitochondria was measured by the method of McGivan et al. (13). Liver mitochondria was prepared according to the method of Novicoff and Hews (14). After hepatic mitochondria (3 mg protein/mL) was preincubated with 2 mM sodium phosphate, 3 mM succinate, 10 mM sucrose, 1 \(\mu\)g/mL of oligomycin, 0.5 mM aminooxyacetate, 225 mM choline chloride and 10 mM Tris-morpholinopropanesulfonate (pH 7.0) at 20\(^\circ\)C for 2 min, 5 mM \(^3\)H-ornithine (18.5 kBq/mL) was added together with \(^1\)C-sucrose (9.25 kBq/mL), which was used as a marker of the extramitochondrial space. After a further 2 min, the pellets and supernatants were separated by centrifugation, acidified and assayed for radioactivity (LS 5000TD, Beckman Japan, Tokyo, Japan). When the effects of amino acids on ornithine transport into mitochondria were investigated, 0.5–5 mM amino acids was added to the reaction mixture.

**Experiment 3.** The effects of thyroid hormone treatment in vivo and the addition of lysine in vitro on ornithine transport into isolated mitochondria in the liver were studied. The same experimental design as in Experiment 1 was used. To
determine the effect of lysine on ornithine transport, 2 mM lysine was supplemented.

**Analytical procedures.** The plasma and urinary concentrations of urea were measured by the method of Archibald (15). The 3,5,3'-triiodo-L-thyronine concentration in plasma was measured by RIA (16). For measuring the concentration of free lysine and ornithine, the liver was treated with ice-cold 2% sulfosalicylic acid to precipitate the protein (17). Lysine and ornithine were measured using an amino acid analyzer (L-8500, Hitachi, Tokyo, Japan).

**Statistical analysis.** The means and pooled SEM were reported. Duncan's multiple range test was used to compare means after one-way ANOVA (18, 19). Data on ornithine transport into hepatic mitochondria were analyzed by Duncan's multiple range test after two-way ANOVA. A linear regression analysis was used to assess the relationship between the ornithine transport into hepatic mitochondria and urinary excretion of urea (19). Differences were considered significant at \( p < 0.05 \).

**RESULTS**

**Experiment 1**

The effects of thyroid hormone on the plasma concentration of urea, urea excretion in urine and the hepatic concentrations of ornithine and lysine are presented in Table 2. The PTU + T₃ treatment caused a significantly lower body weight gain measured over the 5-d T₃ or saline treatment period than in rats treated 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 body weight. Compared with rats given PTU without T₃ treatment, the plasma concentration of T₃ was significantly higher in rats given PTU + T₃ and the control rats. Urinary excretion, plasma concentration of urea and hepatic concentration of ornithine in rats treated with PTU alone were

|                      | Control | PTU | PTU + T₃ | Pooled SEM |
|----------------------|---------|-----|----------|------------|
| Body weight gain (g/5 d) | 16.3ᵃ   | 12.1ᵇ  | 5.5ᶜ    | 0.6        |
| Liver weight (g/100 g body wt) | 4.40    | 4.33  | 4.22    | 0.10       |
| Urinary urea (mmol/d)  | 5.39ᵇ   | 7.23ᵃ  | 4.72ᶜ   | 0.15       |
| Plasma urea (mmol/L)  | 5.02ᵇ   | 7.94ᵃ  | 3.71ᶜ   | 0.19       |
| Plasma T₃ (nmol/L)    | 1.72ᵇ   | 0.63ᶜ  | 9.70ᵃ   | 0.29       |
| Liver ornithine (µmol/g liver) | 0.349ᵇ  | 0.505ᵃ | 0.309ᶜ  | 0.013      |
| Liver lysine (µmol/g liver)   | 0.625ᵇ  | 0.392ᶜ | 1.301ᵃ  | 0.028      |

¹ Values are mean and pooled SEM of six rats. Means with different superscript letters are significantly different \( (p < 0.05) \). T₃, triiodothyronine.

² Body weight gain only during the 5 d of T₃ or saline treatment is presented.
significantly greater than in rats given PTU+T₃ or the control rats. The liver concentration of lysine in rats treated with PTU alone was significantly reduced compared to rats given PTU+T₃ or the control rats.

**Experiment 2**

Table 3 shows the effects of amino acids *in vitro* on ornithine transport into hepatic isolated mitochondria. The addition of non-essential amino acids to the incubation mixture did not affect ornithine transport. However, only lysine, among all the essential amino acids, suppressed the rate of ornithine transport in a dose-dependent manner. With 2 mM lysine, a 30% inhibition in ornithine transport was observed.

**Experiment 3**

The effects of thyroid hormone treatment *in vivo* and the addition of lysine *in vitro* on the urinary excretion of urea and ornithine transport into isolated mitochondria in liver are presented in Table 4. As in Experiment 1, the group receiving PTU+T₃ grew less over the 5-d of T₃ treatment than the group given PTU alone. The excretion of urea in rats treated with PTU alone was significantly higher than in rats given PTU+T₃ or the control rats. Whether or not lysine was added *in vitro*, the treatment with thyroid hormone affected ornithine transport into

| Concentration of amino acids (mM) | Ornithine transport (nmol/mg protein) | % Inhibition |
|-----------------------------------|--------------------------------------|--------------|
| Control                           | 1.54                                 | 0            |
| + N.E.A. As²                      | 1.56                                 | 0            |
| + Methionine                      | 1.67                                 | 0            |
| + Threonine                       | 1.68                                 | 0            |
| + Isoleucine                      | 1.62                                 | 1            |
| + Leucine                         | 1.61                                 | 2            |
| + Valine                          | 1.68                                 | 0            |
| + Tryptophan                      | 1.66                                 | 0            |
| + Phenylalanine                   | 1.62                                 | 1            |
| + Lysine                          | 1.16                                 | 29           |
| + Histidine                       | 1.57                                 | 4            |
| Control                           | 1.60                                 |              |
| + Lysine                          | 0.5                                  | 4            |
| + Lysine                          | 1                                    | 14           |
| + Lysine                          | 2                                    | 29           |
| + Lysine                          | 5                                    | 30           |

¹ Results are averages of triplicate experiments.  
² Non-essential amino acids.
Table 4. Effects of thyroid hormone treatment in vivo and lysine treatment in vitro on ornithine transport into isolated hepatic mitochondria and urinary excretion of urea (Experiment 3).  

|                         | Body weight gain\(^2\) (g/5 d) | Liver weight (g/100 g body wt) | Urinary urea (mmol/d) | Ornithine transport |
|-------------------------|-------------------------------|-------------------------------|-----------------------|--------------------|
|                         |                               |                               |                       | Basal              |
| Control                 | 16.5\(^{a}\)                 | 4.45                          | 5.36\(^{b}\)          | 1.65\(^{b}\)       |
| PTU                     | 12.8\(^{b}\)                 | 4.31                          | 7.11\(^{a}\)          | 1.91\(^{a}\}       |
| PTU + T\(_{3}\)         | 5.6\(^{c}\)                  | 4.20                          | 4.67\(^{c}\)          | 1.12\(^{c}\)       |
| Pooled SEM              | 0.7                           | 0.12                          | 0.17                  | 0.06               |

ANOVA  
Thyroid hormone  
Lysine  
Thyroid hormone × Lysine

\(^{1}\) Values are mean and pooled SEM of six rats. *Significantly different from corresponding value in rats of basal group at p < 0.05. The superscript letters indicate significant differences of means (p < 0.05) due to type of thyroid hormone treatment within basal or lysine treatment groups. NS, not significant (p > 0.05).  
T\(_{3}\), triiodothyronine.

\(^{2}\) Body weight gain only during the 5 d of T\(_{3}\) or saline treatment is presented.

isolated hepatic mitochondria. Compared with the group treated with PTU + T\(_{3}\) or the controls, ornithine transport into mitochondria was significantly greater in the PTU-treated group. There was a significant positive correlation between ornithine transport into hepatic mitochondria and urinary excretion of urea (r = 0.907, p < 0.001). The addition of lysine in vitro caused a decrease in ornithine transport in all groups. This decrease was statistically significant. The thyroid hormone × lysine interaction for ornithine transport into hepatic mitochondria was not significant.

DISCUSSION

In a previous study (3), we found that PTU treatment caused a higher excretion of urinary urea but lower activities of urea cycle enzymes than those of the control group, and that the treatment of PTU-treated rats with T\(_{3}\) tended to reverse the effects of PTU. The purpose of these experiments was to elucidate the mechanism by which thyroid hormone reduces urea synthesis. The regulation of urea synthesis by thyroid hormone may not be attributable to changes in the activities of urea cycle enzymes (4). In this study, we tried to determine whether or not ornithine transport into hepatic mitochondria might regulate urea synthesis when thyroid hormone status is manipulated. The active form of thyroid hormone, T\(_{3}\), was administered. The dose of T\(_{3}\) was relatively high [0.1 mg/(100 g body wt · d)].

Ornithine was an intermediate of the urea cycle. Katunuma et al. (20)
emphasized that an increased hepatic ornithine concentration could be involved in activating the urea cycle under the conditions of varying dietary level of protein. Urea formation has been shown to be stimulated by the addition of ornithine during liver perfusion (7, 8). However, as ornithine transcarbamylase is distributed in hepatic mitochondria, ornithine synthesized by arginase must be transported into the mitochondria. The Km-value (1.8 mM) of ornithine transcarbamylase for ornithine was reported to be markedly higher than the mitochondrial ornithine concentration (21). Zollner (12) suggested that citrulline formation was dependent on the rate of ornithine transport into hepatic mitochondria in hepatocytes when ammonia was supplied, and that the transport of ornithine limited the rate of urea production. An indication for limitation of urea synthesis by ornithine transport in vivo was reported by Saheki et al. (22). The injection of ammonium salt to rats fed a low-protein diet decreased the mitochondrial ornithine concentration in liver. Urea synthesis showed only a very small response. In contrast, when animals were fed a high-protein diet, the ornithine concentration and urea production increased. Therefore, we assumed that higher urea production in the PTU-treated group might be due to the elevation of ornithine transport into hepatic mitochondria. In this study, the greater ornithine transport into mitochondria of the PTU-treated group enhanced urea synthesis in the group (Table 4). In previous reports (3, 4, 10), we demonstrated that a greater hepatic concentration of ornithine in hypothyroid rats might be one of the factors regulating urea production when thyroid status is manipulated. The changes in not only ornithine concentration but also ornithine transport into mitochondria in liver may have regulated urea synthesis in this study.

Ornithine transport is inhibited by lysine treatment in isolated mitochondria (13) and hepatocytes (12). Therefore, we assumed that lysine concentration regulates ornithine transport into mitochondria. In this study, from all amino acids, treatment with lysine in vitro only significantly inhibited ornithine transport. This response was observed well within the physiological concentration of liver lysine (Tables 3 and 4). Zollner (12) investigated the effects of several amino acids (alanine, glycine, glutamic acid, lysine, leucine, valine and isoleucine) on ornithine transport in hepatocytes, and demonstrated that only lysine decreased the rate of ornithine transport. These results clearly indicate the presence of a transport system for ornithine, which seems to be specifically inhibited by lysine under physiological conditions. The hepatic concentration of free lysine in rats given PTU without T3 treatment was markedly lower than in the control rats or rats treated with PTU+T3 (Table 2). The lower concentration of liver lysine may induce to greater ornithine transport into mitochondria in the PTU-treated group. The regulation of urea synthesis may be partly mediated through the inhibition of ornithine transport by lysine when thyroid status is manipulated. Many investigators indicated (12, 13) that ornithine was transported into mitochondria on a specific carrier. McGivan et al. (13) suggested that lysine abolished the binding of ornithine to the outer surface (e.g., the binding to the ornithine carrier) of the mitochondrial membrane because lysine did not penetrate the membrane. In this
study as well, the ornithine carrier system might have been suppressed by the lysine treatment.

When lysine was not added in vitro, thyroid hormone treatment in vivo also decreased ornithine transport into mitochondria (Table 3). Therefore, the thyroid hormone may affect not only the regulation of ornithine transport mediated by the lysine concentration but also ornithine transport directly. However, little documentation of the direct effect of thyroid hormones on the carrier system for ornithine transport is available. This is another possibility to consider in further examination of the mechanism by which thyroid hormones alter urea synthesis.

The mechanism by which thyroid hormone status affects the hepatic concentration of lysine remains to be determined. Thyroid hormones are known to induce protein synthesis in visceral organs and skeletal muscles (3,16,23). In one of our previous reports (24), however, the hepatic concentrations of many amino acids were the lowest in the group treated with PTU alone, and the liver activities of aspartate transaminase (EC 2.6.1.1), alanine transaminase (EC 2.6.1.2) and serine dehydratase (EC 4.2.1.13) were the highest in this group. Further studies should seek to determine whether lysine catabolizing enzymes may control the hepatic concentration of lysine when thyroid status is changed.

Rats receiving a high dose of T₃ exhibited less body weight gain than rats treated with PTU alone or controls (Table 2). However, lower excretions of urea and nitrogen were observed in T₃-treated rats (24). When animals were given T₃ in an earlier study (3), we showed that the carcass concentration of lipids was significantly lower and that of proteins was significantly higher. The hyperlipidemia of hypothyroidism and hypolipidemia of hyperthyroidism are well-accepted clinical findings (25,26). The reduction in carcass fat concentration seems to be associated with reduced growth following T₃ treatment.

The results suggest that the greater ornithine transport into mitochondria in the hypothyroid (PTU alone) rats is likely to stimulate urea synthesis. Thyroid hormone-induced increase in lysine concentration may be at least partly responsible for the changes in ornithine transport.

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