Decreased Activity and Expression of Intestinal Oligopeptide Transporter PEPT1 in Rats with Hyperthyroidism *in Vivo*

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Purpose. To examine the effect of thyroid hormone status on PEPT1 *in vivo*, the activity and expression of PEPT1 in the small intestine were examined in euthyroid and hyperthyroid rats.

Methods. Hyperthyroidism was induced by treating rats with L-thyroxine (12 mg/L) in the drinking water for 21 days. Transport activity was measured by everted small intestinal preparations and *in situ* intestinal loop technique. Expressions of PEPT1 mRNA and protein were evaluated by competitive polymerase chain reaction and Western blotting, respectively.

Results. The uptake of [^{14}C]glycylsarcosine by everted small intestinal preparations was significantly decreased in hyperthyroid rats, whereas that of methyl-α-D-[^{14}C(U)]-glucopyranoside was not altered. Kinetic analysis showed that the V_{max} value for [^{14}C]glycylsarcosine uptake was significantly decreased in hyperthyroid rats, whereas the K_m value was not affected. The mean portal vein concentrations after intrajejunal administration of [^{14}C]glycylsarcosine were also decreased in hyperthyroid rats. Moreover, hyperthyroidism caused a significant decrease in the expression of PEPT1 mRNA in the small intestine, whereas the expression of Na^+/glucose cotransporter (SGLT1) mRNA was not changed. The level of PEPT1 protein was also decreased in the small intestine of hyperthyroid rats.

Conclusions. These results indicate that in hyperthyroid rats, the activity and expression of PEPT1 were decreased in the small intestine.

KEY WORDS: hormonal regulation; hyperthyroidism; intestinal absorption; PEPT1.

INTRODUCTION

Thyroid hormone, produced by the thyroid gland, is essential for normal development, differentiation, metabolic balance, and physiological function of virtually all tissues (1). Disorders of the thyroid gland are among the most common endocrine diseases and are known as hyperthyroidism and hypothyroidism. Hyperthyroidism may be associated with severe dysfunction of the gastrointestinal tract and gastrointestinal symptoms, particularly diarrhea and malabsorption with steatorrhea, well-known symptoms in hyperthyroid patients (2). Therefore, there have been a number of studies investigating the influence of hyperthyroidism on alterations in motility in the gastrointestinal tract (3), structural changes in the small intestine (4,5), and absorption of several nutrients (6). In addition, hyperthyroidism has been shown to affect drug pharmacokinetics (7). The intestinal absorption of nutrients as well as some drugs is mediated by various transporters (8). Therefore, understanding the effect of hyperthyroidism on the function and expression of transporters would be useful for treatment of patients with thyroid disorders.

Transport of di- or tripeptides across plasma membranes in the small intestine plays a pivotal role in efficient absorption of protein digestion products. The absorption process is mediated actively by the H^+/peptide cotransport system localized in the brush-border membrane. H^+-coupled peptide transporter (PEPT1) in the small intestine has been cloned and well characterized (8–14). PEPT1 is localized to the brush-border membrane (15) and mediates the absorption of digestion products of protein and peptide-like drugs such as β-lactam antibiotics, the anti-cancer agent bestatin, angiotensin-converting enzyme inhibitors, as well as L-valyl ester of several drugs such as valacyclovir (8,11–14).

Recently, we demonstrated the effect of thyroid hormone on the activity and expression of PEPT1 in human intestinal epithelial cell line Caco-2 (16). Treatment of Caco-2 cells with 3,5,3′-L-triiodothyronine (T_3) inhibited [^{14}C]glycylsarcosine uptake in a time- and concentration-dependent manner. Moreover, T_3 treatment caused a significant decrease in the levels of PEPT1 mRNA and protein expression, suggesting that T_3 treatment inhibited the uptake of glycylsarcosine by decreasing the transcription and/or stability of PEPT1 mRNA. However, the effect of thyroid hormone on the activity and expression of PEPT1 in intact tissues remains to be elucidated.

In this study, to investigate the effect of thyroid hormone on PEPT1 *in vivo*, we examined the activity and expression of PEPT1 in the small intestine of hyperthyroid rats. The current data indicate that hyperthyroidism *in vivo* caused a decrease in the activity and expression of PEPT1 in the small intestine.

MATERIALS AND METHODS

Materials

[^{14}C]Glycylsarcosine (1.78 GBq/mmol) was obtained from Daiichi Pure Chemicals Co. (Ibaraki, Japan). L-[3-^{3}H]Alanine (1.48 TBq/mmol) and methyl-α-D-[^{14}C(U)]-glucopyranoside (9.66 GBq/mmol) were from Moravek Biochemical (Brea, CA, USA). L-Thyroxine (T_4) was purchased from Sigma (St. Louis, MO, USA). All other chemicals were of the highest purity available.

Treatment of Animals

The animal experiments were performed in accordance with the Guideline for Animal Experiments of Kyoto University. Wistar rats (8 weeks old, 220–280 g body weight) were provided with standard rat chow *ad libitum.* Hyperthyroidism was induced by adding T_4 (12 mg/L) to the drinking water for 21 days according to the method of Weinstein *et al.* (17). T_4 was added as a 6 mg/ml stock solution in 0.1 M NaOH. Euthyroid rats were given drinking water including the same volume of 0.1 M NaOH. After treatment, the small intestine

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ABBREVIATIONS: GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PCR, polymerase chain reaction; PEPT, H^+/peptide cotransporter; SGLT, Na^+/glucose cotransporter; T_3, 3,5,3′-L-triiodothyronine; T_4, L-thyroxine.

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was excised for uptake studies, isolation of total RNA, and preparation of crude plasma membranes. Blood was also taken for measurement of plasma level of T_3. Plasma T_3 levels in euthyroid and hyperthyroid rats were measured by an Enzyme Immuno Assay method (IMx, Dainabot, Tokyo, Japan). Plasma T_3 levels in euthyroid and hyperthyroid rats were 0.38 ± 0.02 and 2.19 ± 0.20 ng/ml, respectively (mean ± SE, n = 20 to 25 rats). In addition, hyperthyroid rats lost weight in response to T_3 treatment (euthyroid, 417.1 ± 6.2 g; hyperthyroid, 366.8 ± 5.2 g).

Uptake Studies by Everted Small Intestinal Preparations

The uptake of [^{14}C]glycylsarcosine was measured in everted segments of the jejunum isolated from euthyroid and hyperthyroid rats as described previously (18) with some modifications. Briefly, the everted intestinal segments were preincubated in incubation buffer (pH 6.5) at 37°C for 5 min. After preincubation, the intestine was immediately placed in a vial containing drug solution. After incubation for an appropriate time, the tissues were washed twice in ice-cold isotonic solution, blotted on filter paper, weighed, and solubilized in 0.5 ml of NCS II (Amersham Biosciences, Buckinghamshire, UK). The radioactivity was determined in 5 ml of ACS II (Amersham Biosciences) by liquid scintillation counting.

In situ Loop Technique

Intestinal absorption of [^{14}C]glycylsarcosine was examined by *in situ* loop technique. A cannula with a polyethylene tube was inserted in the portal vein. A jejunum loop 10 cm in length was prepared, and [^{14}C]glycylsarcosine (40 nmol·ml^{-1}·kg body wt^{-1}) was introduced into the loop with a microsyringe. Blood was withdrawn from the portal vein at designated times. Blood samples were centrifuged for 2 min at 14,000 × *g*, and 50 μl of plasma was solubilized in 0.5 ml of NCS II. The radioactivity was determined in 5 ml of ACS II by liquid scintillation counting.

Competitive Polymerase Chain Reaction

Competitive polymerase chain reaction (PCR) was performed according to the method of Siebert and Larrick (19) with some modifications as described previously (20,21). The specific primer sets used are as follows: sense, 5′-GTGTGGGGCCCCAATCTATACCGT-3′ (bases 1442–1465), antisense, 5′-GTTTGTCTGTGAGACAGGTTCCAA-3′ (bases 2153–2176) for PEPT1; sense, 5′-ATGGACAGTAGCACCTTGAGCC-3′ (bases 170–191), antisense, 5′-TAGCCCCAGAGAAGATGTCTGC-3′ (bases 647–668) for Na^+/glucose cotransporter (SGLT1); sense, 5′-CCTTCATTGACCTCAACTAC-3′ (bases 131–150), antisense, 5′-GGAAGGCCATGCCAGTGAGC-3′ (bases 705–724) for glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Western Blotting

The crude membrane fractions of the small intestine from euthyroid and hyperthyroid rats were prepared as described previously (15). Western blot analysis was performed as described previously (22). Antisera against rat PEPT1 (22) and villin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were used as primary antibodies.

Statistical Analysis

Data were analyzed statistically by nonpaired *t* test or one-way analysis of variance followed by Scheffé's test when multiple comparisons were needed. Probability values less than 5% were considered significant.

RESULTS

Glycylsarcosine, Methyl-α-D-Glucopyranoside, and Alanine Transport Studies

To investigate whether the oligopeptide transport activity was altered in the small intestine of hyperthyroid rats, the uptake of [^{14}C]glycylsarcosine by everted small intestinal preparations was examined. Figure 1 shows the time-course of [^{14}C]glycylsarcosine uptake by everted small intestinal segments. The amount of [^{14}C]glycylsarcosine uptake at each time point by the small intestine of hyperthyroid rats was reduced, compared with that of euthyroid rats. Next, we examined the effect of thyroid hormone status on the uptake of various substrates by everted small intestinal preparations. As shown in Fig. 2, [^{14}C]glycylsarcosine and [^{3}H]alanine uptake by everted small intestinal preparations were significantly reduced in hyperthyroid rats. In contrast, there was no significant difference in methyl-α-D-[^{14}C(U)]-glucopyranoside uptake between euthyroid and hyperthyroid rats. These results indicate that the inhibitory effect of thyroid hormone status

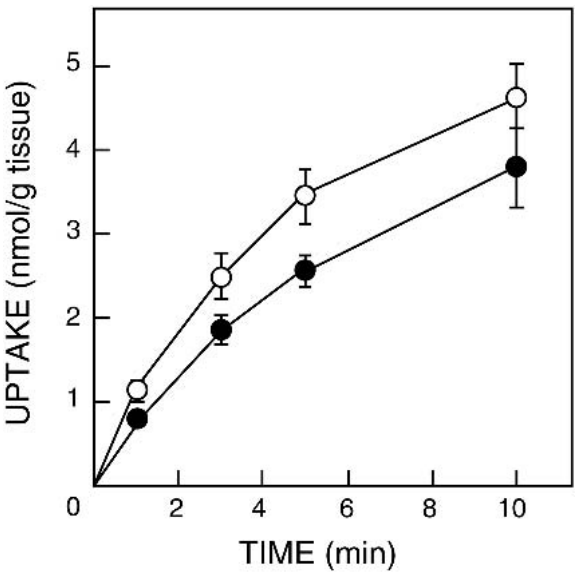

Fig. 1. Time-course of [^{14}C]glycylsarcosine uptake by everted small intestinal preparations isolated from euthyroid or hyperthyroid rats. Hyperthyroidism was induced by treatment with T_4 for 21 days as described in "Materials and Methods." Everted small intestinal preparations from euthyroid (○) or hyperthyroid (●) rats were incubated at 37°C in incubation medium (pH 6.5) containing 5 μM [^{14}C]glycylsarcosine. Thereafter, the radioactivity of the solubilized tissue was determined. Each point represents the mean ± SE of 14 to 16 everted small intestinal segments from 4 rats in 2 separate experiments.

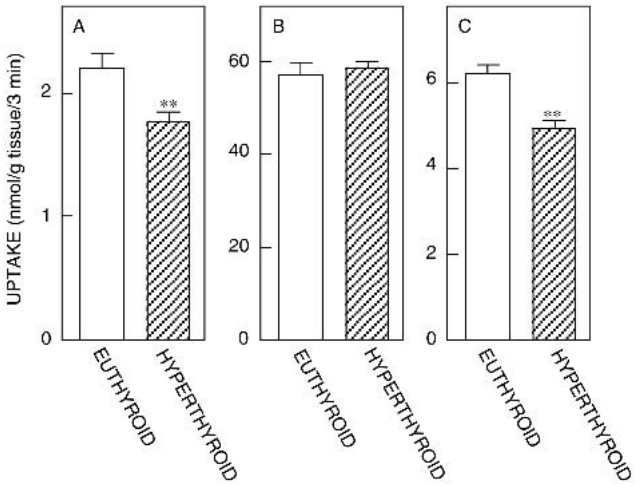

Fig. 2. [^{14}C]Glycylsarcosine, methyl-α-D-[^{14}C(U)]-glucopyranoside, and [^{3}H]alanine uptake by everted small intestinal preparations isolated from euthyroid or hyperthyroid rats. Everted small intestinal preparations from euthyroid (open column) or hyperthyroid (hatched column) rats were incubated with incubation medium containing [^{14}C]glycylsarcosine (5 μM, pH 6.5; A), methyl-α-D-glucopyranoside (0.1 mM, pH 7.4; B) or [^{3}H]alanine (20 μM, pH 7.4; C) for 3 min at 37°C. Thereafter, the radioactivity of the solubilized tissue was determined. Each column represents the mean ± SE of 13 to 31 everted intestinal segments from 3 to 8 rats in two or four separate experiments. **$p < 0.01$, significantly different from euthyroid rats.

on [^{14}C]glycylsarcosine uptake was not due to nonspecific effects.

Kinetic Analysis of Glycylsarcosine Uptake

To determine the effect of thyroid hormone status on the kinetics of [^{14}C]glycylsarcosine uptake, the concentration dependence of [^{14}C]glycylsarcosine uptake was examined in euthyroid or hyperthyroid rats. Figure 3 shows the initial uptake of [^{14}C]glycylsarcosine as a function of the substrate concentration. Specific uptake was calculated by subtracting the nonspecific uptake, which was estimated in the presence of excess unlabeled dipeptide, from total uptake. Kinetic parameters were calculated according to the Michaelis-Menten equation using nonlinear least-squares regression analysis. The apparent K_m values for the uptake of [^{14}C]glycylsarcosine by everted small intestinal preparations from euthyroid and hyperthyroid rats were 4.82 ± 0.52 and 5.21 ± 0.78 mM (mean ± SE of three separate experiments), respectively. The apparent V_{max} values were 1.46 ± 0.12 and 0.77 ± 0.11 μmol·g tissue^{-1}·3 min^{-1} (mean ± SE of three separate experiments, $p < 0.01$), respectively. Thus, hyperthyroidism caused a significant decrease in the apparent V_{max} value for [^{14}C]glycylsarcosine uptake, whereas the apparent K_m value did not change significantly.

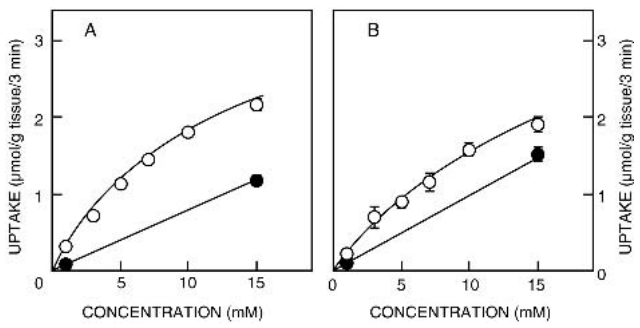

Fig. 3. Concentration dependence of [^{14}C]glycylsarcosine uptake by everted small intestinal preparations from euthyroid or hyperthyroid rats. Everted small intestinal preparations from (A) euthyroid or (B) hyperthyroid rats were incubated with incubation medium containing various concentrations of [^{14}C]glycylsarcosine (pH 6.5) in the absence (○) or presence (●) of 50 mM unlabeled glycylleucine for 3 min at 37°C. Thereafter, radioactivity of solubilized tissue was determined. Each point represents the mean ± SE of 3 everted small intestinal segments from a typical experiment. When error bars are not shown, they are smaller than the symbol.

Glycylsarcosine Absorption by *in situ* Intestinal Loops

We then examined the intestinal absorption of [^{14}C]glycylsarcosine by *in situ* intestinal loops from euthyroid and hyperthyroid rats. Figure 4 shows the mean portal vein concentrations after intrajejunal administration of [^{14}C]glycylsarcosine. The appearance of [^{14}C]glycylsarcosine in the portal vein was significantly slower in hyperthyroid rats than that in euthyroid rats.

Competitive PCR Analysis

The decrease in the V_{max} value for [^{14}C]glycylsarcosine uptake by everted small intestinal preparations from hyperthyroid rats suggested the decreased expression of the peptide transporter PEPT1 in the rat small intestine. Therefore, we then carried out competitive PCR analysis to obtain quantitative information about the level of PEPT1 mRNA expression. The data obtained by competitive PCR amplification using specific primer sets were normalized with the data of competitive PCR for GAPDH in each tissue (data not shown). Representative results of agarose-gel electrophoresis

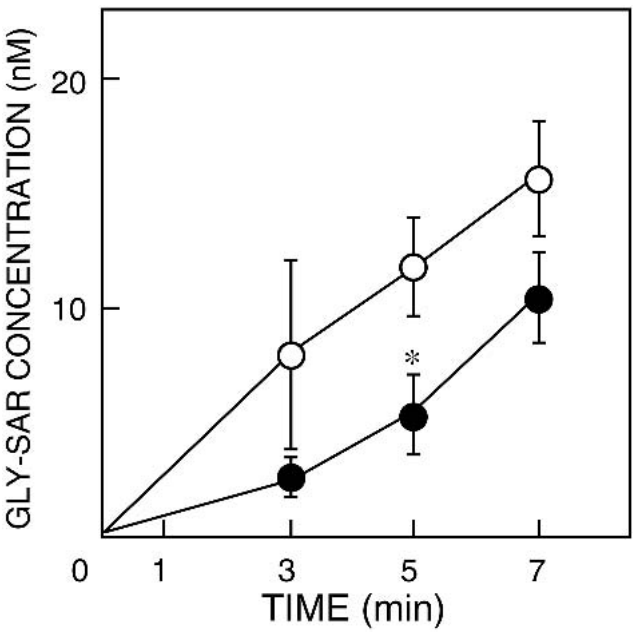

Fig. 4. Time-course of portal vein [^{14}C]glycylsarcosine (GLY-SAR) concentration by *in situ* intestinal loop experiments from euthyroid (○) and hyperthyroid (●) rats. Each points represents the mean ± SE of 6–11 rats. *$p < 0.05$, significantly different from euthyroid rats.

are shown in Fig. 5A. The relative band density in each reaction was determined densitometrically, and the amount of mRNA was quantified. The amount of competitor yielding equal molar amounts of products gave the initial amount of target. As shown in Fig. 5B, the expression level of PEPT1 mRNA in the small intestine of hyperthyroid rats was significantly decreased, compared with that of euthyroid rats. However, the expression level of SGLT1 mRNA in the small intestine of hyperthyroid rats was not different from that of euthyroid rats.

Western Blotting

Western blotting was performed to determine the level of PEPT1 protein expression in the small intestine. Crude membranes from the small intestine were subjected to immunoblot analysis using anti-rat PEPT1 and anti-villin antibodies (Fig. 6A). The arrowheads indicate the position of each protein. To obtain quantitative data of the expression level of PEPT1, the densitometric level of PEPT1 was normalized for each membrane by correcting the densitometric level of villin as an internal standard. As shown in Fig. 6B, the level of PEPT1 protein expression in the small intestine of hyperthyroid rats was significantly decreased (70% of the control).

DISCUSSION

Thyroid hormone is an important regulator of gut mucosal growth, differentiation, and barrier function. For example, in both suckling and adult rats, T_3 is known to be a trophic factor for intestinal crypt cells (4,23). In addition, T_3 regulates the levels of several brush-border enzymes (24). The absorption of some nutrients and minerals was also shown to be affected by thyroid state (6). The occurrence of diarrhea and steatorrhea in hyperthyroidism was well documented, and it has been reported that these symptoms occurred due to the alteration in motility of gastrointestinal tract in hyperthyroidism. The motor activity of the gastrointestinal tract is increased in hyperthyroidism and decreased in hypothyroidism (3,6). On the other hand, the absorption of some nutrients and minerals is mediated by specific transporters. The alteration of the activity of transporters by thyroid hormone might effect on the absorption of some nutrients and minerals. Indeed, Matosin-Matekalo *et al.* reported that

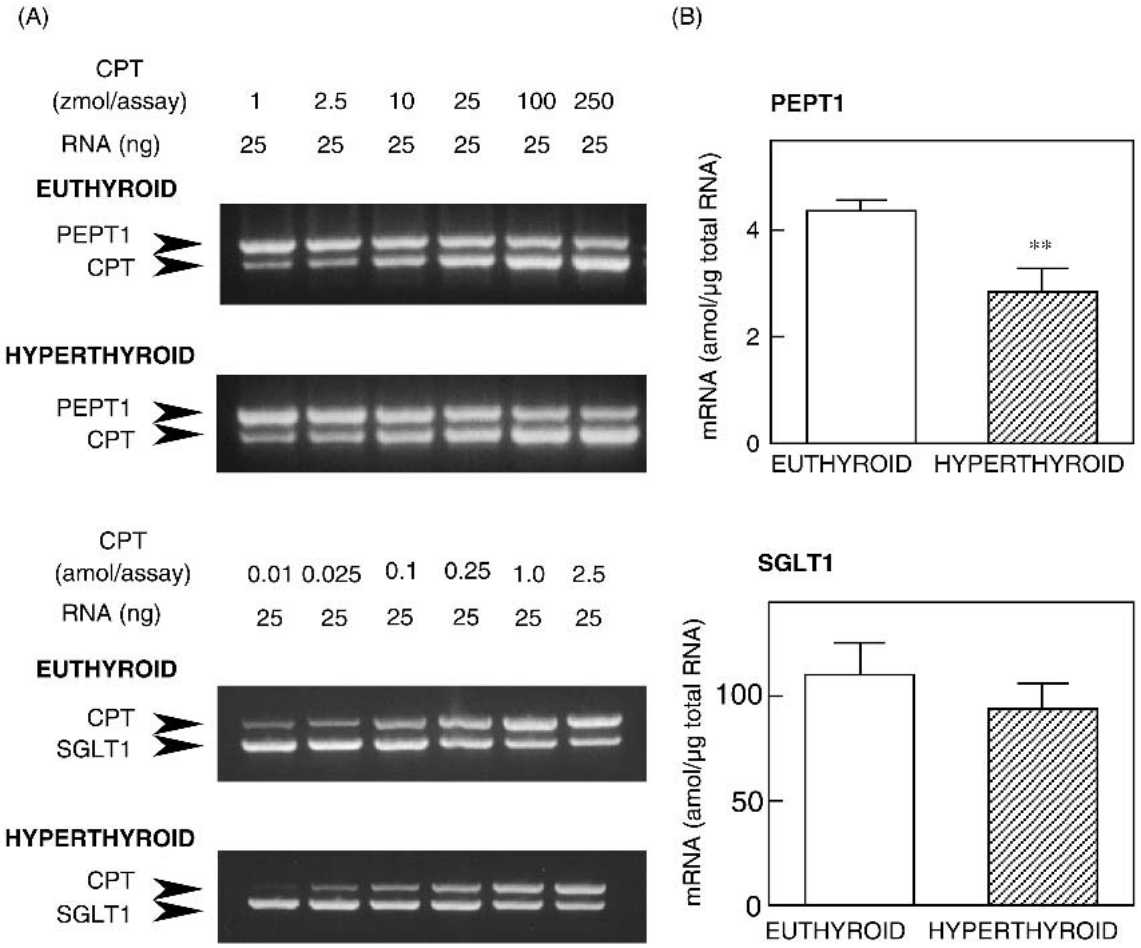

Fig. 5. Quantification of levels of PEPT1 and SGLT1 mRNA expression in total RNA isolated from the small intestine of euthyroid and hyperthyroid rats. (A) PCR amplification was carried out as described in "Materials and Methods." These photographs show the results of representative experiments. CPT, competitor. (B) Columns indicate the levels of PEPT1 and SGLT1 mRNA expression in the small intestine from euthyroid (open column) or hyperthyroid (hatched column) rats determined densitometrically. Each bar represents the mean ± SE of seven rats. $^{**}p < 0.01$, significantly different from euthyroid rats.

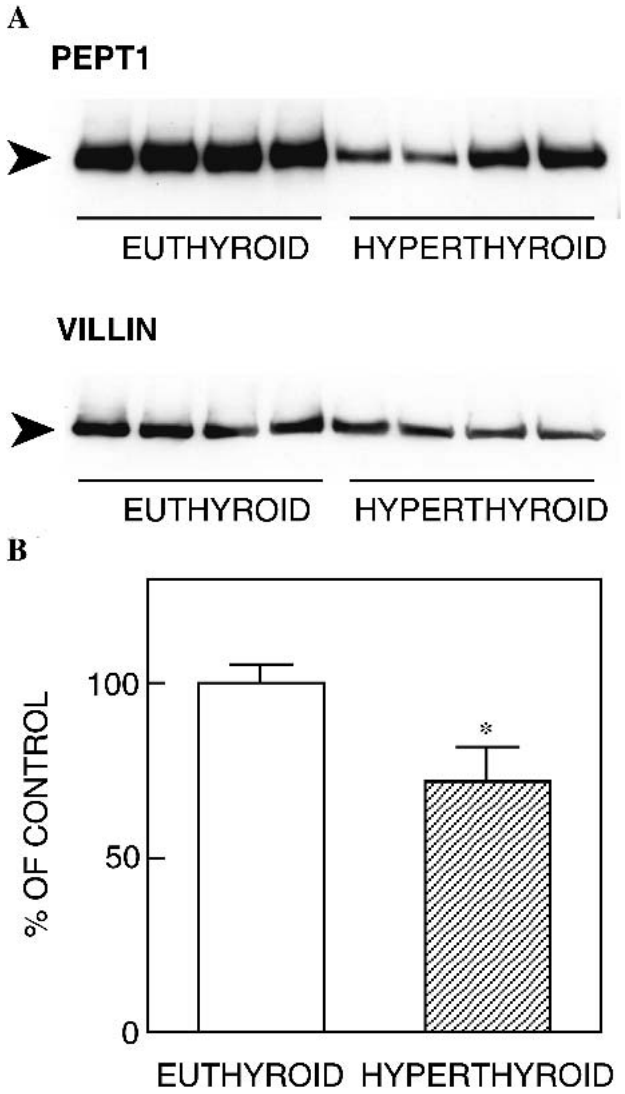

Fig. 6. Western blot analysis of intestinal crude membranes from euthyroid or hyperthyroid rats for PEPT1. (A) Membranes (50 μg) from each tissue were separated by 8.5% SDS-PAGE, and antisera specific for rat PEPT1 (1:2000 dilution) and villin (1:1000) were used as primary antibodies. The arrowheads indicate the positions of PEPT1 and villin. (B) Densitometric quantification of PEPT1, corrected using the amount of villin. Euthyroid control level was set at 100%. Each column represents the mean ± SE of nine rats from three separate experiments. *$p < 0.05$, significantly different from euthyroid controls.

T_3 treatment stimulated α-methylglucose uptake via SGLT1 in Caco-2/TC7 cells (25). Therefore, we hypothesized that the absorption of oligopeptides, protein digestion products, via PEPT1 might be affected by the state of thyroid hormone.

Recently, we examined the effect of thyroid hormone on the activity and expression of PEPT1 in human intestinal Caco-2 cells (16). Treatment of Caco-2 cells with T_3 decreased the activity and expression of PEPT1. In the current study, to investigate the effect of thyroid hormone on PEPT1 *in vivo*, we examined the activity and expression of PEPT1 in hyperthyroid rats. The current results showed that [^{14}C]glycylsarcosine uptake by everted small intestinal preparations and the level of PEPT1 protein expression in the small intestine were significantly decreased in hyperthyroid rats. The level of PEPT1 mRNA was also decreased in hyperthyroid rats, indicating that thyroid hormone might act on PEPT1 mRNA expression directly or indirectly. In contrast, the uptake of methyl-α-D-[^{14}C(U)]-glucopyranoside and the expression level of SGLT1 mRNA were not altered in hyperthyroid rats. These results are incompatible with the report of Matosin-Matekalo *et al.* in which increased expression of SGLT1 mRNA was demonstrated in Caco-2/TC7 cells treated with T_3 (25). In addition, earlier work by Bronk and Parsons (26) also demonstrated that treatment of thyroidectomized rats with T_3 increased the mucosal accumulation of galactose, a substrate of SGLT1, though thyroidectomy itself had no effect on galactose accumulation. However, the effect of thyroid hormone on the expression of SGLT1 *in vivo* has not been reported so far. Therefore, it seems likely that these discrepancies are due to the difference in the experimental conditions.

Thyroid gland secretes two very different types of hormones: T_3 and T_4. However, most of the hormone released is T_4, and most of the T_3 circulating in the blood is derived from peripheral metabolism of T_4. In this study, hyperthyroid rats were induced by adding T_4 to the drinking water for 21 days as reported previously (17). The administration of T_4 produces both hormones, because T_4 is converted to T_3 intracellularly. For thyroid replacement and suppression therapy, T_4 is selected because of its stability, content uniformity, lack of allergenic foreign protein, and long half-life (7 days). Although the concentration of T_3 is lower than that of T_4 in the plasma, the action of T_3 is three to four times more potent than that of T_4 and it is considered to be an active form of thyroid hormone. Therefore, we measured the concentration of T_3 in the plasma to certify the induction of hyperthyroidism. The concentration of T_3 was higher in hyperthyroid rats than euthyroid (2.19 ± 0.20 vs. 0.38 ± 0.02 ng/ml). The concentration of T_3 in this study was similar to other studies (17,27). In addition, the concentration of T_3 in patients with hyperthyroidism was about 2 to 5 times higher than in normal subjects. Therefore, hyperthyroidism induced in this study may correlate with pathological hyperthyroidism in humans.

Hyperthyroidism is the clinical syndrome that results when tissues are exposed to high levels of thyroid hormone. The influence of hyperthyroidism on drug pharmacokinetics has been reviewed by O'Connor and Feely (7) and Shenfield (28). Such pharmacokinetic changes suggest that individualization and proper dosage of some drugs is necessary in patients with hyperthyroidism. The peptide transporter PEPT1 plays an important role in the absorption of peptide-like drugs such as β-lactam antibiotics, bestatin, renin inhibitor, angiotensin-converting enzyme inhibitors, and valacyclovir (8,11–14). Therefore, the alteration of the activity and expression of PEPT1 in the small intestine in hyperthyroidism might induce changes in absorption of such drugs. We thus studied the effect of hyperthyroidism on the absorption of [^{14}C]glycylsarcosine from intestine to portal vein with *in situ* loop technique and demonstrated that the appearance of [^{14}C]glycylsarcosine in the portal vein was significantly decreased in hyperthyroid rats. These results suggest that the absorption of peptide-like drugs is changed in thyroid disorders.

Regulation of PEPT1 in the small intestine has widely been studied (8,12,13). Short-term regulation of peptide transport involves either modulation of the intrinsic activity of transporter molecules or membrane insertion of additional transporter molecules recruited from a preexisting intracellu-

lar pool. For example, it has been reported that insulin (29), the α_{2A}-adrenergic agonist clonidine (30), and leptin (31) appeared to increase the translocation of PEPT1 from a preformed cytoplasmic pool to the plasma membrane. In addition, Anderson *et al.* demonstrated that short-term treatment of Caco-2 cells with vasoactive intestinal peptide or pituitary adenylate cyclase-activating polypeptide inhibited the uptake of glycylsarcosine indirectly through inhibition of the apical Na^+/H^+ exchanger NHE3 (32). In contrast, it was reported that long-term treatment with several factors altered the levels of PEPT1 mRNA and/or protein expression. Thamotharan *et al.* (33) and Walker *et al.* (34) reported that dipeptides up-regulated the activity of PEPT1 in Caco-2 cells, accompanied by increases in the levels of PEPT1 mRNA and protein expression. Shiraga *et al.* (35) showed that the up-regulation of dipeptide transport activity by dietary protein was caused by transcriptional activation of the rat PEPT1 gene by selective amino acids and dipeptides in the diet. Starvation for 4 days caused an increase in the amount of PEPT1 protein, whereas administration of amino acids decreased the expression of PEPT1 protein (36). Long-term epidermal growth factor treatment of Caco-2 cells caused a decrease in the transport of glycylsarcosine, a decrease in total human PEPT1 protein contents, and a decrease in human PEPT1 mRNA level (37). In our study, long-term treatment with thyroid hormone caused a decrease in the activity of PEPT1, caused by the decreased levels of PEPT1 mRNA and protein expression in the small intestine. To our knowledge, this is the first report showing hormonal down-regulation of the activity and expression of PEPT1 *in vivo*.

In conclusion, we found that hyperthyroidism *in vivo* caused a decrease in the activity and expression of PEPT1 in the small intestine. These findings provide useful information for the maintenance of protein nutrition and optimization of pharmacotherapy with peptide-like drugs in patients with hyperthyroidism.

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REFERENCES

1. P. M. Yen. Physiological and molecular basis of thyroid hormone action. *Physiol. Rev.* **81**:1097–1142 (2001).
2. W. R. J. Middleton. Thyroid hormones and the gut. *Gut* **12**:172–177 (1971).
3. M. Wegener, B. Wedmann, T. Langhoff, J. Schaffstein, and R. Adamek. Effect of hyperthyroidism on the transit of a caloric solid-liquid meal through the stomach, the small intestine, and the colon in man. *J. Clin. Endocrinol. Metab.* **75**:745–749 (1992).
4. A. J. Wall, W. R. J. Middleton, A. G. E. Pearse, and C. C. Booth. Intestinal mucosal hyperplasia following induced hyperthyroidism in the rat. *Virchows Arch. B Cell Pathol.* **6**:79–87 (1970).
5. R. A. Hodin, A. Shei, M. Morin, and S. Meng. Thyroid hormone and the gut: selective transcriptional activation of a villus-enterocyte marker. *Surgery* **120**:138–143 (1996).
6. R. J. Levin. The effects of hormones on the absorptive, metabolic and digestive functions of the small intestine. *J. Endocrinol.* **45**: 315–348 (1969).
7. P. O'Connor. and J. Feely. Clinical pharmacokinetics and endocrine disorders. Therapeutic implications. *Clin. Pharmacokinet.* **13**:345–364 (1987).
8. T. Katsura and K. Inui. Intestinal absorption of drugs mediated by drug transporters: mechanisms and regulation. *Drug Metab. Pharmacokin.* **18**:1–15 (2003).
9. F. H. Leibach and V. Ganapathy. Peptide transporters in the intestine and the kidney. *Annu. Rev. Nutr.* **16**:99–119 (1996).
10. S. A. Adibi. The oligopeptide transporter (Pept-1) in human intestine: biology and function. *Gastroenterology* **113**:332–340 (1997).
11. K. Inui and T. Terada. Dipeptide transporters. In G. L. Amidon and W. Sadée (eds.), *Membrane Transporters as Drug Targets*, Kluwer Academic/Plenum, New York, 1999 pp. 269–288.
12. D. Meredith and C. A. R. Boyd. Structure and function of eukaryotic peptide transporters. *Cell. Mol. Life Sci.* **57**:754–778 (2000).
13. B. Brodin, C. U. Nielsen, B. Steffansen, and S. Frøkjær. Transport of peptidomimetic drugs by the intestinal di/tri-peptide transporter, PepT1. *Pharmacol. Toxicol.* **90**:285–296 (2002).
14. I. Rubio-Aliaga and H. Daniel. Mammalian peptide transporters as targets for drug delivery. *Trends Pharmacol. Sci.* **23**:434–440 (2002).
15. H. Ogihara, H. Saito, B.-C. Shin, T. Terada, S. Takenoshita, Y. Nagamachi, K. Inui, and K. Takata. Immuno-localization of H^+/peptide cotransporter in rat digestive tract. *Biochem. Biophys. Res. Commun.* **220**:848–852 (1996).
16. K. Ashida, T. Katsura, H. Motohashi, H. Saito, and K. Inui. Thyroid hormone regulates the activity and expression of the peptide transporter PEPT1 in Caco-2 cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* **282**:G617–G623 (2002).
17. S. P. Weinstein, E. O'Boyle, M. Fisher, and R. S. Haber. Regulation of GLUT2 glucose transporter expression in liver by thyroid hormone: evidence for hormonal regulation of the hepatic glucose transport system. *Endocrinology* **135**:649–654 (1994).
18. X. Pan, T. Terada, M. Irie, H. Saito, and K. Inui. Diurnal rhythm of H^+-peptide cotransporter in rat small intestine. *Am. J. Physiol. Gastrointest. Liver Physiol.* **283**:G57–G64 (2002).
19. P. D. Siebert and J. W. Larrick. Competitive PCR. *Nature* **359**: 557–558 (1992).
20. H. Motohashi, S. Masuda, T. Katsura, H. Saito, S. Sakamoto, S. Uemoto, K. Tanaka, and K. Inui. Expression of peptide transporter following intestinal transplantation in the rat. *J. Surg. Res.* **99**:294–300 (2001).
21. K. Takahashi, S. Masuda, N. Nakamura, H. Saito, T. Futami, T. Doi, and K. Inui. Upregulation of H^+-peptide cotransporter PEPT2 in rat remnant kidney. *Am. J. Physiol. Renal Physiol.* **281**:F1109–F1116 (2001).
22. H. Saito, M. Okuda, T. Terada, S. Sasaki, and K. Inui. Cloning and characterization of a rat H^+/peptide cotransporter mediating absorption of β-lactam antibiotics in the intestine and kidney. *J. Pharmacol. Exp. Ther.* **275**:1631–1637 (1995).
23. K. Yeh and F. Moog. Development of the small intestine in the hypophysectomized rats. *Dev. Biol.* **47**:173–184 (1975).
24. S. J. Henning. Postnatal development: coordination of feeding, digestion, and metabolism. *Am. J. Physiol.* **241**:G199–G214 (1981).
25. M. Matosin-Matekalo, J. E. Mesonero, O. Delezay, J. C. Poiree, A. A. Ilundain, and E. Brot-Laroche. Thyroid hormone regulation of the Na^+/glucose cotransporter SGLT1 in Caco-2 cells. *Biochem. J.* **334**:633–640 (1998).
26. J. R. Bronk and D. S. Parsons. Influence of the thyroid gland on the accumulation of sugars in rat intestinal mucosa during absorption. *J. Physiol. (Lond.)* **179**:323–332 (1965).
27. S. Euzet, M. Lelièvre-Pégorier, and C. Merlet-Bénichou. Effect of 3,5,3′-triiodothyronine on maturation of rat renal phosphate transport: kinetic characteristics and phosphate transporter messenger ribonucleic acid and protein abundance. *Endocrinology* **137**:3522–3530 (1996).
28. G. M. Shenfield. Influence of thyroid dysfunction on drug pharmacokinetics. *Clin. Pharmacokinet.* **6**:275–297 (1981).
29. M. Thamotharan, S. Z. Bawani, X. Zhou, and S. A. Adibi. Hormonal regulation of oligopeptide transporter Pept-1 in a human intestinal cell line. *Am. J. Physiol.* **276**:C821–C826 (1999).
30. F. Berlioz, J. J. Maoret, H. Paris, M. Laburthe, R. Farinotti, and C. Rozé. α_2-Adrenergic receptors stimulate oligopeptide transport in a human intestinal cell line. *J. Pharmacol. Exp. Ther.* **294**:466–472 (2000).
31. M. Buyse, F. Berlioz, S. Guilmeau, A. Tsocas, T. Voisin, G. Pér-

anzi, D. Merlin, M. Laburthe, M. J. M. Lewin, C. Rozé, and A. Bado. PepT1-mediated epithelial transport of dipeptides and cephalexin is enhanced by luminal leptin in the small intestine. *J. Clin. Invest.* **108**:1483–1494 (2001).
32. C. M. H. Anderson, M. E. Mendoza, D. J. Kennedy, D. Raldua, and D. T. Thwaites. Inhibition of intestinal dipeptide transport by the neuropeptide VIP is an anti-absorptive effect *via* the $VPAC_1$ receptor in a human enterocyte-like cell line (Caco-2). *Br. J. Pharmacol.* **138**:564–573 (2003).
33. M. Thamotharan, S. Z. Bawani, X. Zhou, and S. A. Adibi. Mechanism of dipeptide stimulation of its own transport in a human intestinal cell line. *Proc. Assoc. Am. Physicians* **110**:361–368 (1998).
34. D. Walker, D. T. Thwaites, N. L. Simmons, H. J. Gilbert, and B. H. Hirst. Substrate upregulation of the human small intestinal peptide transporter, hPepT1. *J. Physiol. (Lond.)* **507**:697–706 (1998).
35. T. Shiraga, K. Miyamoto, H. Tanaka, H. Yamamoto, Y. Taketani, K. Morita, I. Tamai, A. Tsuji, and E. Takeda. Cellular and molecular mechanisms of dietary regulation on rat intestinal H^+/peptide transporter PepT1. *Gastroenterology* **116**:354–362 (1999).
36. H. Ogihara, T. Suzuki, Y. Nagamachi, K. Inui, and K. Takata. Peptide transporter in the rat small intestine: ultrastructural localization and the effect of starvation and administration of amino acids. *Histochem. J.* **31**:169–174 (1999).
37. C. U. Nielsen, J. Amstrup, B. Steffansen, S. Frokjaer, and B. Brodin. Epidermal growth factor inhibits glycylsarcosine transport and hPepT1 expression in a human intestinal cell line. *Am. J. Physiol. Gastrointest. Liver Physiol.* **281**:G191–G199 (2001).