Life Sciences 116 (2014) 74–82

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Effects of triiodothyronine on turnover rate and metabolizing enzymes for thyroxine in thyroidectomized rats

Hidenori Nagao [a,*], Makoto Sasaki [a], Tetsuya Imazu [a], Kenjo Takahashi [b], Hironori Aoki [a], Kouichi Minato [a]

[a] Pharmacokinetics Research Department of ASKA Pharmaceutical Co., Ltd., Kawasaki, Japan
[b] Department of Analytical Research, ASKA Pharma Medical Co., Ltd., Kawasaki, Japan

ARTICLE INFO

Article history:
Received 11 June 2014
Accepted 13 September 2014
Available online 28 September 2014

Keywords:
Thyroid hormones
Isotope tracer
On-line SPE LC-MS/MS
Turnover rate
Thyroidectomy
Deiodinases
UDP-glucuronosyltransferases
Sulfotransferases

ABSTRACT

Aim: Previous studies in rats have indicated that surgical thyroidectomy represses turnover of serum thyroxine (T_4). However, the mechanism of this process has not been identified. To clarify the mechanism, we studied adaptive variation of metabolic enzymes involved in T_4 turnover.
Main methods: We compared serum T_4 turnover rates in thyroidectomized (Tx) rats with or without infusion of active thyroid hormone, triiodothyronine (T_3). Furthermore, the levels of mRNA expression and activity of the metabolizing enzymes, deiodinase type 1 (D1), type 2 (D2), uridine diphosphate-glucuronosyltransferase (UGT), and sulfotransferase were also compared in several tissues with or without T_3 infusion.
Key findings: After the T_3 infusion, the turnover rate of serum T_4 in Tx rats returned to normal. Although mRNA expression and activity of D1 decreased significantly in both liver and kidneys without T_3 infusion, D2 expression and activity increased markedly in the brain, brown adipose tissue, and skeletal muscle. Surprisingly, hepatic UGT mRNA expression and activity in Tx rats increased significantly in comparison with normal rats, and returned to normal after T_3 infusion.
Significance: This study suggests that repression of the disappearance of serum T_4 in rats after Tx is a homeostatic response to decreased serum T_3 concentrations. Additionally, T_4 glucuronide is a storage form of T_4, but may also have biological significance. These results suggest strongly that repression of deiodination of T_4 by D1 in the liver and kidneys plays a major role in thyroid hormone homeostasis in Tx rats, and that hepatic UGT also plays a key role in this mechanism.

Introduction

Pro-thyroid hormone, thyroxine (T_4) is the main secretory product of the thyroid gland, and serum T_4 is primarily metabolized by deiodinases and conjugation enzymes.

Deiodinases consist of three types. Type 1 deiodinase (D1) catalyzes both outer ring deiodination (ORD) and inner ring deiodination (IRD) in the liver, kidney, pituitary gland, and thyroid (Chopra, 1977; Green, 1978). Type 2 deiodinase (D2) only catalyzes ORD in the central nervous system, brown adipose tissue (BAT), and skeletal muscle (Silva et al., 1982; Silva and Larsen, 1983; Marsili et al., 2010). The ORD of T_4 is the only way to produce active thyroid hormone, triiodothyronine (T_3) and is an important activating pathway. Biological activity of T_3 is three to four times more potent than that of T_4. Type 3 deiodinase (D3) only catalyzes IRD in the brain, skin, and placenta (Kaplan et al., 1983; Huang et al., 1985). This enzyme catalyzes the conversion of T_4 to inactive reverse T_3 (Gereben et al., 2008).

Conjugation of the phenolic hydroxyl group with glucuronic acid or sulfuric acid is an important metabolic pathway. Both conjugates are considered to be biologically inactive and have increased water solubility, facilitating urinary and biliary excretion. Uridine diphosphate-glucuronosyltransferases (UGTs) are classified into two families, UGT1 and UGT2, in both rats and humans (Tukey and Strassburg, 2000). In rat UGT1 family, UGT1A1 and UGT1A6 are important isoforms responsible for the glucuronidation of T_4 in liver and kidneys (Vansell and Klaassen, 2002). Sulfotransferases (SULTs) consist of three families, phenol SULTs, estrogen SULTs, and hydroxysteroid SULTs (Weinshilboum et al., 1997; Falany, 1997). Sulfation of iodothyronines is catalyzed by phenol SULTs (SULT1A1, 1B1, and 1C1) in the liver and kidneys (Dunn and Klaassen, 2000). In male rat liver, SULT1C1 is almost exclusively expressed (Liu and Klaassen, 1996; Yamazoe et al., 1994).

We previously reported that serum T_4 and T_3 levels in rats were decreased but not completely abolished after surgical Tx. These hormone levels were maintained at a constant very low level throughout the experimental period, and the turnover rate of T_4 in Tx rats was slower than in normal rats (Nagao et al., 2011). These findings suggest that the disappearance of serum T_4 is suppressed by Tx, and serum T_4 is supplied by extra-thyroidal tissues (e.g. secretion of extra-thyroidal storage, enhancement of enterohepatic recirculation, and production

* Corresponding author at: Pharmacokinetics Research Department of ASKA Pharmaceutical Co., Ltd., 5-36-1, Shimosakunobe, Takatsu-ku, Kawasaki 213-8522, Japan. Tel.: +81 44 812 8656; fax: +81 44 822 1265.
E-mail address: nagao-h@aska-pharma.co.jp (H. Nagao).

http://dx.doi.org/10.1016/j.lfs.2014.09.016
0024-3205/

in extra-thyroidal tissues). In contrast, although serum T_4 levels of iodine-deficient rats are decreased similar to in Tx rats, T_3 levels are normal (Minato et al., 2012). Furthermore, there were no differences in turnover rates of T_4 among iodine-deficient and normal rats. These findings suggested that prolongation of the half-life of serum T_4 after Tx is a homeostatic response to stabilize thyroid hormone activity to decreased serum T_3 concentrations. However, the homeostatic mechanisms of thyroid hormone activity are complex, and many details remain unknown.

The aim of the present study was to clarify the mechanism of repression of serum T_4 degradation after Tx. We evaluated turnover rates of serum T_4 in Tx rats infused with T_3 using a stable isotope-labeled tracer ($[^{13}C_9]T_4$) method (Nagao et al., 2011). Furthermore, we investigated the mRNA expression and activity of T_4 metabolizing enzymes (D1, D2, UGTs, and SULTs) in several tissues in Tx rats infused with or without T_3.

Materials and methods

Chemicals

T_4, T_3 and reverse T_3 (rT_3) were purchased from Sigma-Aldrich Co. (NJ, USA). L-Thyroxine-[L-tyrosine-$^2H_5$]HCl ($[^2H_5]T_4$) was purchased from IsoSciences, LLC (King of Prussia, PA, USA). $[^{13}C_9]$ T_4 was synthesized chemically from $[^{13}C_9]$ tyrosine in our laboratory (Nagao et al., 2011). $[^{125}I]T_4$ and $[^{125}I]rT_3$ were obtained from PerkinElmer (MA, USA). They were purified on Sephadex LH-20 (Pharmacia, Uppsala, Sweden) just before each assay (Rutgers et al., 1989). All other chemicals and reagents were of the highest analytical grade commercially available.

Experimental animals

Seven-week-old male Sprague–Dawley rats were obtained from Charles River Laboratories Japan (Kanagawa, Japan). Animals were fed a commercial diet (FR-2, Funabasi Farm Co., Chiba, Japan) and filtered tap water ad libitum. The cages were located in a light (0800–2000 h lights on), temperature (23 ± 5 °C) and humidity (60 ± 20%) controlled room. The rats were allowed to acclimatize for 1 week before starting the experiments.

All experimental procedures were approved by the Animal Research Committee of ASKA Pharmaceutical Co., in accordance with the Basic Guidelines for Proper Conduct of Animal Testing and Related Activities in the Research Institutions under the Jurisdiction of the Ministry of Health, Labour and Welfare of Japan.

Thyroidectomy

Rats were made hypothyroid by surgical thyroidectomy as described previously in detail (Nagao et al., 2011). After the surgery, serum TSH rapidly increased and, body weight gain was complete stasis. Complete resection of the thyroid in the Tx rats was confirmed at the end of the experiment by macroscopic observation at necropsy. In addition, serial sections from tracheal tubes (area of thyroid glands in Tx rats) were reviewed by pathologists and thyroid tissues including follicular epithelial cells were not observed.

Experiment 1

At 3 weeks after Tx treatments, 15 Tx rats were divided into 3 groups (n = 5 each). Osmotic minipumps (Alzet, model 2ML4, DURECT, CA, USA), delivering 1.5 μg T_3/head/day (Tx + T3 rats; n = 5) or 7.5 μg T_3/head/day (Tx + HT3 rats; n = 5) were implanted subcutaneously on day 0 under isoflurane anesthesia. The doses of T_3 were determined by our preliminary experiment and previous literature (Nguyen et al., 1993). The vehicle was saline, in which T_3 (final concentration; 25 μg/ml in 0.5 mM NaOH/saline) was diluted after NaOH solubilization (5 mg T_3/20 μl of 1 M NaOH). Five sham-operated (control) and Tx rats received pumps with 0.5 mM NaOH/saline only. On day 14 after osmotic minipump implantation, $[^{13}C_9]T_4$ was administered intravenously to all group rats at a dose of 1.5 μg/500 μl/kg (Nagao et al., 2011). On the day before pump implantation (day −1), day 13, and days 14–23 (for 10 days after $[^{13}C_9]T_4$ injection), serum samples were collected from tail vein. All samples were obtained between 9 and 11 a.m. to minimize fluctuations in thyroid hormones (Jeremiah et al., 1990) and stored at −20 °C until analyzed.

Experiment 2

Osmotic minipumps, delivering 1.5 μg T_3/head/day (Tx + T3 rats; n = 6) were implanted subcutaneously under isoflurane anesthesia. Six control and Tx rats received pumps with 0.5 mM NaOH/saline only. On day 14 after the implantation, all rats were exsanguinated via a needle in the abdominal aorta. The liver, kidneys, brain, brown adipose tissue (BAT), and thigh muscles (skeletal muscles) were dissected and processed immediately frozen in liquid nitrogen and stored at −80 °C until measured for enzyme activity. Aliquots of these tissues were stored at −20 °C in Allprotect tissue reagent (QIAGEN, Venlo, Netherlands) until analyzed for RT-PCR.

Serum thyroid hormone concentrations

T_3, T_4, and $[^{13}C_9]T_4$ were determined by the method using on-line SPE LC-MS/MS (Nagao et al., 2011). In brief, an API5000 triple–quadrupole mass spectrometer (AB SCIEX, CA, U.S.A.) equipped with a TurboIonSpray source and Shimadzu HPLC system was employed to perform the analysis using isotope dilution with deuterium labeled internal standard, $[^2H_5]T_4$. A 20 μl aliquot of rat serum was mixed with internal standard acetonitrile solution for deproteinization, and was diluted with 0.1 vol.% formic acid. After centrifugation, the supernatant was injected into the LC-MS/MS system. On-line extraction and chromatographic separation of the analytes were performed using respectively a Shim pack MAYI-ODS, 2.0 mm i.d. × 10 mm, 50 μm (Shimadzu, Kyoto, Japan) and a Synergi Polar-RP 80A, 2.0 mm i.d. × 50 mm, 4 μm (Phenomenex, Utrecht, the Netherlands). Details of the LC conditions including gradient elution and the selected reaction monitoring transitions are described in a previous report (Nagao et al., 2011). Linear calibration curves of T_3, T_4, and $[^{13}C_9]$ T_4 were obtained in the concentration range of 0.1–100 ng/ml, with a lower limit of quantitation of 0.1 ng/ml.

Tissue preparations

Frozen tissues (liver, kidneys, brain, BAT and skeletal muscles) were pulverized, and the obtained powder was suspended in 5 volumes ice-cold preparation buffer (20 mM Tris–HCl(pH7.6), 0.25 mM sucrose, 1.2 mM EDTA, 5 mM dithiothreitol (DTT, Wako Pure Chemical Industries, Osaka, Japan)), and complete protease inhibitor cocktail (Roche, Basel, Switzerland). Homogenization was accomplished using a Tissue Tearor (BiospecProducts, OK, USA). The homogenates were centrifuged at 1000 ×g at 4 °C for 15 min to remove any remaining particle, and stored at −80 °C until analyzed except the skeletal muscles. The homogenate of the skeletal muscles was used for the following process without freeze preservation. Aliquots of the homogenates of liver, kidneys and skeletal muscles were centrifuged at 10,000 ×g at 4 °C for 20 min. The supernatants were centrifuged at 105,000 ×g at 4 °C for 1 h, and the microsomal pellets of the liver and kidneys were resuspended in ice-cold preparation buffer. Microsomes (liver and kidneys) and cytosol (skeletal muscles) were stored at −80 °C until analyzed. Protein levels of tissue fractions were measured by the method of Lowry et al. (1951), using BSA as a reference.