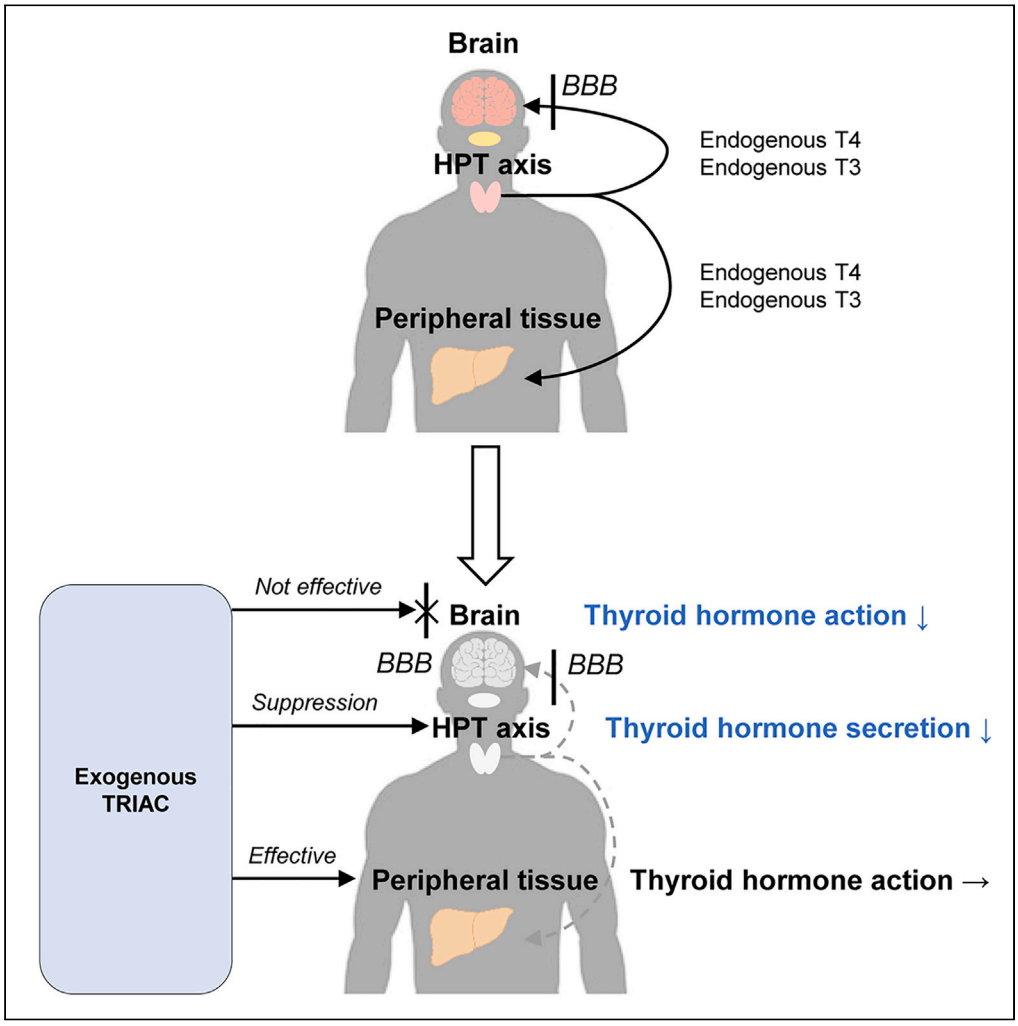
iScience

CellPress
OPEN ACCESS

Article

TRIAC disrupts cerebral thyroid hormone action via negative feedback and heterogenous distribution among organs



Ichiro Yamauchi, Takuro Hakata, Yohei Ueda, ..., Daisuke Nakajima, Takuya Kubo, Nobuya Inagaki

ichiroy@kuhp.kyoto-u.ac.jp

Highlights

TRIAC administration does not upregulate thyroid hormone action in the cerebrum

TRIAC is not efficiently trafficked into the cerebrum but suppresses the HPT axis

Via the combined effects, TRIAC administration depletes cerebral thyroid hormones

Yamauchi et al., iScience 26, 107135
July 21, 2023
https://doi.org/10.1016/j.isci.2023.107135

iScience

CellPress
OPEN ACCESS

Article

TRIAC disrupts cerebral thyroid hormone action via negative feedback and heterogenous distribution among organs

Ichiro Yamauchi,[1,6,*] Takuro Hakata,[1] Yohei Ueda,[1] Taku Sugawa,[1] Ryo Omagari,[2] Yasuo Teramoto,[2] Shoji F. Nakayama,[2] Daisuke Nakajima,[2,3] Takuya Kubo,[4] and Nobuya Inagaki[1,5]

SUMMARY

As 3,3′,5-triiodothyroacetic acid (TRIAC), a metabolite of thyroid hormones (THs), was previously detected in sewage effluent, we aimed to investigate exogenous TRIAC's potential for endocrine disruption. We administered either TRIAC or 3,3′,5-triiodo-L-thyronine (LT3) to euthyroid mice and 6-propyl-2-thiouracil-induced hypothyroid mice. In hypothyroid mice, TRIAC administration suppressed the hypothalamus-pituitary-thyroid (HPT) axis and upregulated TH-responsive genes in the pituitary gland, the liver, and the heart. We observed that, unlike LT3, TRIAC administration did not upregulate cerebral TH-responsive genes. Measurement of TRIAC contents suggested that TRIAC was not efficiently trafficked into the cerebrum. By analyzing euthyroid mice, we found that cerebral TRIAC content did not increase despite TRIAC administration at higher concentrations, whereas serum levels and cerebral contents of THs were substantially decreased. Disruption by TRIAC is due to the additive effects of circulating endogenous THs being depleted via a negative feedback loop involving the HPT axis and heterogeneous distribution of TRIAC among different organs.

INTRODUCTION

Thyroid hormones (THs) are essential for key biological processes, including development, growth, differentiation, and homeostasis. In many species, the hypothalamus-pituitary-thyroid (HPT) axis plays a substantial role in regulating TH action.[1] In brief, thyroid stimulating hormone (TSH) secreted from the pituitary gland promotes the synthesis and secretion of THs by the thyroid gland. TSH production is stimulated by thyrotropin-releasing hormone (TRH) from the hypothalamus. THs inhibit TSH and TRH secretion via a negative feedback loop. In addition to the HPT axis, various components are necessary for TH action, including transporters, iodothyronine deiodinases (DIO1, DIO2, and DIO3), thyroid hormone receptors (THRs), and clearance pathways such as glucuronidation.

The two major THs that bind to and activate THRs are 3,3′,5,5′-tetraiodothyronine (T4) and 3,3′,5-triiodothyronine (T3). T3 is considered the active form because it is more potent than T4 at activating THRs. T3 is secreted from the thyroid gland along with T4 but most circulating T3 is the product of T4 deiodination by DIO1 and DIO2.[2] Meanwhile, some TH metabolites can be detected in human sera, including 3,3′-diiodothyronine, 3,3′,5′-triiodothyronine, 3,3′,5-triiodothyroacetic acid (TRIAC, also known as TA3), and 3,3′,5,5′-tetraiodothyroacetic acid.[3] Among these metabolites, TRIAC is well known for its robust biological activity. TRIAC has a similar affinity to T3 for THRα1 and greater affinity than T3 for THRβ1 and THRβ2.[4–6] TRIAC exerts its effects on the HPT axis as well as other organs, including the heart, bones, and liver of animals as reviewed elsewhere.[7]

Endocrine-disrupting chemicals (EDCs) are exogenous chemicals that interfere with the normal function of hormones.[8] EDCs pose an adverse risk to human health while also impacting natural ecosystems and wildlife. Since people may be exposed to EDCs throughout their entire life, the management of environmental EDCs is crucial.[9] EDCs can disrupt TH actions through a variety of mechanisms. For example, bisphenol A disrupts by acting on THRs,[10] a TH transporter MCT8,[11] and DIO3.[12] A second example is the lowering of circulating TH levels by polychlorinated biphenyls, polybrominated diphenyl ethers, as well as phthalates.[1]

[1]Department of Diabetes, Endocrinology and Nutrition, Graduate School of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan

[2]Health and Environmental Risk Division, National Institute for Environmental Studies, Tsukuba, Ibaraki 305-8506, Japan

[3]Graduate School of Pharmaceutical Sciences, Chiba University, Chiba 260-8675, Japan

[4]Department of Material Chemistry, Graduate School of Engineering, Kyoto University, Nishikyo-ku, Kyoto 615-8530, Japan

[5]Medical Research Institute KITANO HOSPITAL, PIIF Tazuke-kofukai, Osaka 530-8480, Japan

[6]Lead contact

*Correspondence: ichiroy@kuhp.kyoto-u.ac.jp

https://doi.org/10.1016/j.isci.2023.107135

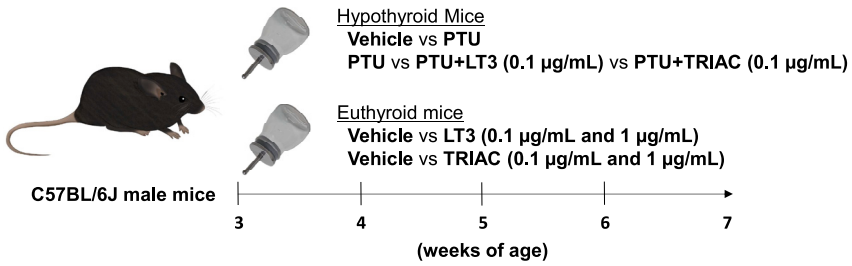


Figure 1. Scheme for generating mouse cohorts

"Hypothyroid" means thyroid hormone synthesis is inhibited by 6-propyl-2-thiouracil (PTU), while "euthyroid" means the absence of inhibition. PTU was administered at a fixed concentration of 100 μg/mL. LT3, 3,3′,5-triiodo-L-thyronine; TRIAC, 3,3′,5-triiodothyroacetic acid. See also Figure S1.

Perchlorate inhibition of iodide uptake by the thyroid gland is a third example of such disruption.[13] Thus, the disruption mechanisms of EDCs are both varied and complex, and must be determined for each EDC.

Our previous work highlighted TRIAC's potential to act as an EDC. THR agonist activity in sewage effluent has been determined in multiple countries.[14–17] We determined TRIAC is a main contributor of THR agonist activity in sewage effluents.[18] Importantly, sewage treatment plants can release chemicals into environmental water, which can subsequently be consumed by humans, as well as wildlife. We reemphasize that TRIAC binds to THRs as a ligand and exhibits agonist activity equal to or greater than that of T3.[4–7] The potential of exogenous TRIAC for endocrine disruption should be urgently investigated to determine its risk to human health.

The aim of the study presented here was to elucidate the impact of exogenous TRIAC on TH action. We administered TRIAC to mice and analyzed the animals using our methods to evaluate various elements that regulate TH action.[19–21] To determine TRIAC's effects, we administered 3,3′,5-triiodo-L-thyronine (LT3) to other mice for comparison. We thereby determined that exogenous TRIAC appears to attenuate TH actions exclusively in the cerebrum.

RESULTS

TRIAC administration to hypothyroid mice

THs are essential to skeletal growth as evidenced by the phenotype of THRβ knockout mice.[22] Since the growth rate of C57BL/6 mice slows considerably after six weeks of age,[23] we determined the effects of THs on growth and other processes by administering LT3 and TRIAC beginning at three weeks of age immediately after weaning (Figure 1). We administered LT3 and TRIAC to euthyroid mice without impairment of TH secretion. Although, we administered 0.1 μg/mL of either LT3 and TRIAC as a supplemental dose or 1 μg/mL as a high dose to the mice, we did not observe significant differences in growth curves based on body weight and naso-anal length except for the naso-anal length of mice administered with 1 μg/mL TRIAC (Figure S1).

To increase the sensitivity of our mouse model for determining TH actions, we induced hypothyroidism in mice using 6-propyl-2-thiouracil (PTU), an anti-thyroid drug that inhibits TH secretion.[24] To determine an optimal concentration, we administered 10 μg/mL or 100 μg/mL of PTU to the mice (Figure S2). PTU decreased serum free T4 levels and enlarged the thyroid gland in a dose-dependent manner (Figures S2A–S2C). However, a marked increase in pituitary *Tshb* (encoding TSH beta subunit) mRNA abundance and significant growth retardation were only observed in mice administered with 100 μg/mL PTU (Figures S2D–S2H). Thus, we administered 100 μg/mL PTU to generate hypothyroid mice. The hypothyroid mice we generated suffered growth retardation as age-dependent increases in body weight and naso-anal length were attenuated (Figures 2A–2D). This attenuation was not due to PTU toxicity but to weakened TH actions because growth retardation was rescued by co-administration of 0.1 μg/mL of either LT3 or TRIAC (Figures 2A–2D). Shortening of the lumbar spine and tibia was also restored by co-administration of either LT3 or TRIAC, which supports the involvement of PTU-induced growth retardation in skeletal growth (Figures 2E–2H).

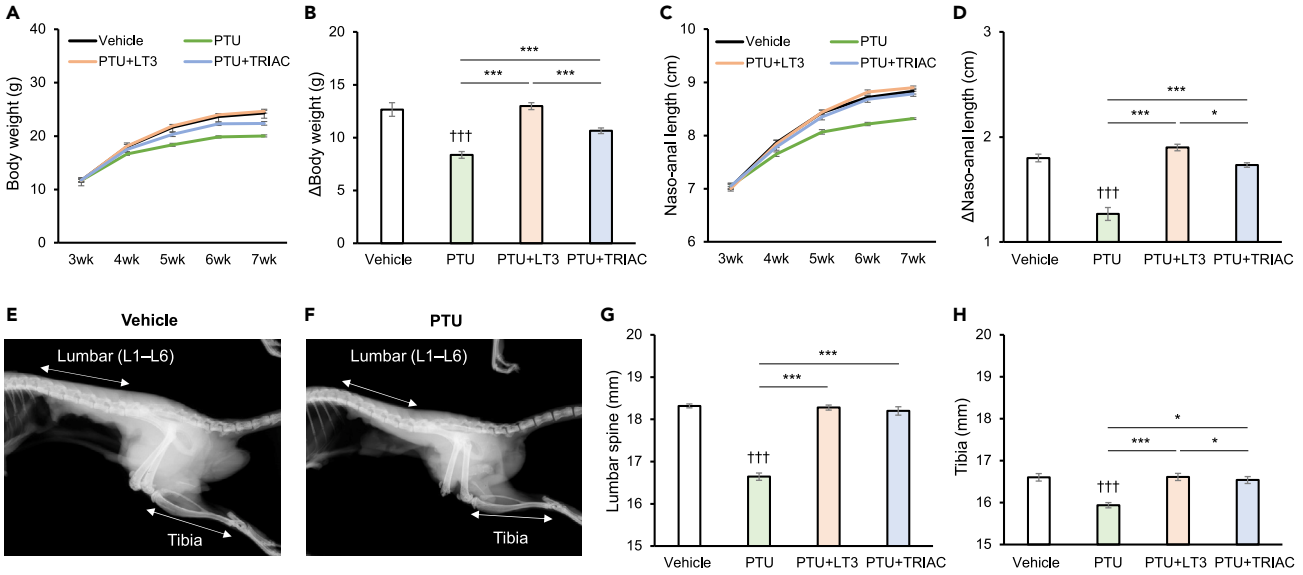


Figure 2. TRIAC effects on growth in hypothyroid mice

(A–D) Growth curves of hypothyroid mice co-administered with LT3 and TRIAC based on body weights (A) and naso-anal lengths (C), and relative amounts of change (Δ) (B, D). Vehicle, PTU, PTU + TRIAC, n = 6 each; and PTU + LT3, n = 5.

(E and F) Soft X-ray images of mice administered with vehicle (E) and PTU (F) at seven weeks of age.

(G and H) Bone lengths of hypothyroid mice at seven weeks of age measured on the soft X-ray images. Concentrations of PTU, LT3, and TRIAC used were 100 μg/mL, 0.1 μg/mL, and 0.1 μg/mL, respectively. Data are represented as means ± SEM. Statistical analyses were performed using Student's *t*-test for vehicle vs. PTU and the p value is presented as †††$p < 0.001$. One-way analysis of variance (ANOVA) followed by the Tukey-Kramer test was used for comparisons among PTU, PTU + LT3, and PTU + TRIAC experiments and the p value is presented as *$p < 0.05$ and ***$p < 0.001$. See also Figure S2 and Tables S1 and S2.

The changes we observed in gross appearance suggest that hypothyroid mice are more suitable for determining TRIAC effects than euthyroid mice. We measured serum TH levels in hypothyroid mice and found that PTU administration decreased serum T4 and T3 levels, while LT3 co-administration increased serum T3 levels and TRIAC co-administration increased serum TRIAC levels (Figures 3A–3C). As for the HPT axis, PTU administration elevated serum TSH levels, which was feedback reaction to low serum T4 and T3 levels (Figure 3D). Furthermore, we found that PTU administration enlarged the thyroid gland (Figures 3E and 3F). Based on histological appearance, follicular cells thickened whereas follicle size decreased under PTU-induced TSH stimulation (Figures 3G and 3H). These changes were fully abrogated by LT3 and reversed to a lesser extent by TRIAC co-administration (Figures 3I and 3J). These results indicate that TRIAC can affect the growth and the HPT axis in a similar manner to T3 even though TRIAC co-administration resulted in weaker changes compared with LT3 co-administration. The intake of LT3 and TRIAC were monitored with amount of water consumption (Table S1): the mice with LT3 co-administration and those with TRIAC co-administration identically drunk 5.1 mL/day at the end of the treatment. Even though we did not protect from light exposure, recovery rates of LT3 and TRIAC in drinking water after 3 days were 108.6% and 103.2%, respectively (Table S2). As water consumption and recovery rate were equivalent, the weaker changes with TRIAC co-administration seemed to be due to shorter biological half-life of TRIAC compared to T3.[25,26]

To investigate differences between TRIAC and T3 with greater sensitivity, we measured the transcript levels of TH-responsive genes and regulators of TH action. As for the pituitary gland, an increase in *Tshb* mRNA following PTU administration was abrogated by co-administering either LT3 or TRIAC (Figure 4A), which corresponded to changes in serum TSH levels (Figure 3D). PTU administration increased *Dio2* mRNA that upregulates TH action by generating T3 from 5′-deiodination of T4. On the other hand, PTU administration decreased *Dio3* mRNA that downregulates TH action by generating reverse T3 from 5-deiodination of T4 and 3,3′-diiodothyronine from 5-deiodination of T3 (Figure 4A). Both changes were restored by co-administering either LT3 or TRIAC (Figure 4A).

We subsequently measured mRNA levels in the liver, heart, and cerebrum. Liver mRNA levels of the TH-responsive genes *Thrsp*, *Idh3a*, and *Dio1* increased following LT3 co-administration and to a lesser extent,

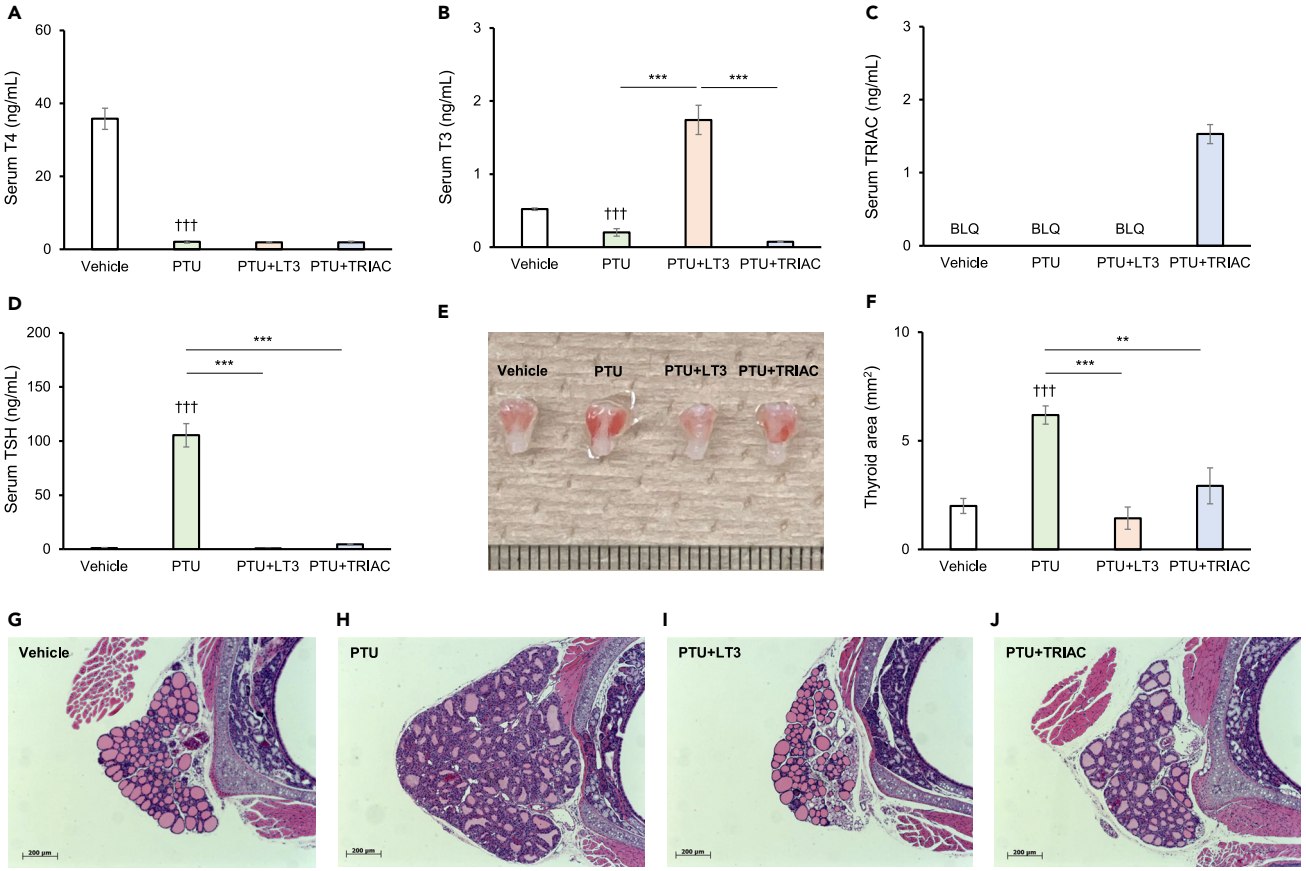


Figure 3. TRIAC effects on the hypothalamus-pituitary-thyroid axis in hypothyroid mice

(A–D) Serum levels of 3,3′,5,5′-tetraiodothyronine (T4), 3,3′,5-triiodothyronine (T3), TRIAC, and thyroid-stimulating hormone (TSH).

(E) Gross appearance of thyroid glands. The scale on the ruler is 1 mm.

(F) Thyroid gland size was measured as thyroid area on photograph.

(G–J) Histological images of thyroid glands stained using hematoxylin and eosin. Vehicle, PTU, PTU + TRIAC, n = 6 each; PTU + LT3, n = 5. BLQ, below the limit of quantitation. Concentrations of PTU, LT3, and TRIAC used were 100 μg/mL, 0.1 μg/mL, and 0.1 μg/mL, respectively. Data are represented as means ± SEM. Statistical analyses were performed using Student's *t*-test for vehicle vs. PTU and the p value is presented as †††$p < 0.001$. ANOVA followed by the Tukey-Kramer test was used for comparisons among PTU, PTU + LT3, and PTU + TRIAC experiments and the p value is presented as **$p < 0.01$, and ***$p < 0.001$.

following TRIAC co-administration (Figure 4B). Considering that attenuation of DIO1 action by PTU was via inhibition of its enzyme activity,[27] increases in *Dio1* mRNA with co-administration of either LT3 or TRIAC and PTU were not peculiar. In the heart, mRNA levels of the TH-responsive genes changed following PTU administration; levels of *Myh6*, *Atp2a2*, and *Hcn2* were lower while *Myh7* levels were higher (Figure 4C). As for *Hcn2* and *Myh7*, these changes were reversed by co-administering LT3 and to a lesser extent, following TRIAC co-administration. PTU administration also decreased *Dio3* mRNA levels in the heart (Figure 4C). TRIAC co-administration reversed this change in *Dio3* mRNA abundance while LT3 co-administration increased *Dio3* mRNA abundance.

Unlike the liver and heart, we observed differences between TRIAC and T3's effects on the cerebrum. Cerebral mRNA levels of *Hr*, *Nrgn*, and *Aldh1a1*, the TH-responsive genes, were decreased following PTU administration; this decrease was reversed by LT3 co-administration but not by TRIAC co-administration (Figure 4D). Additionally, *Dio3* mRNA levels were unchanged following TRIAC co-administration but were significantly higher following LT3 co-administration (Figure 4D). We verified consistency of individual results by normalizing against *Ppia* and *Hprt* (Table S3).

Based on our gene expression analysis, the cerebrum does not respond to TRIAC co-administration. To elucidate the underlying mechanisms, we measured TH contents in each organ. In the liver, PTU decreased

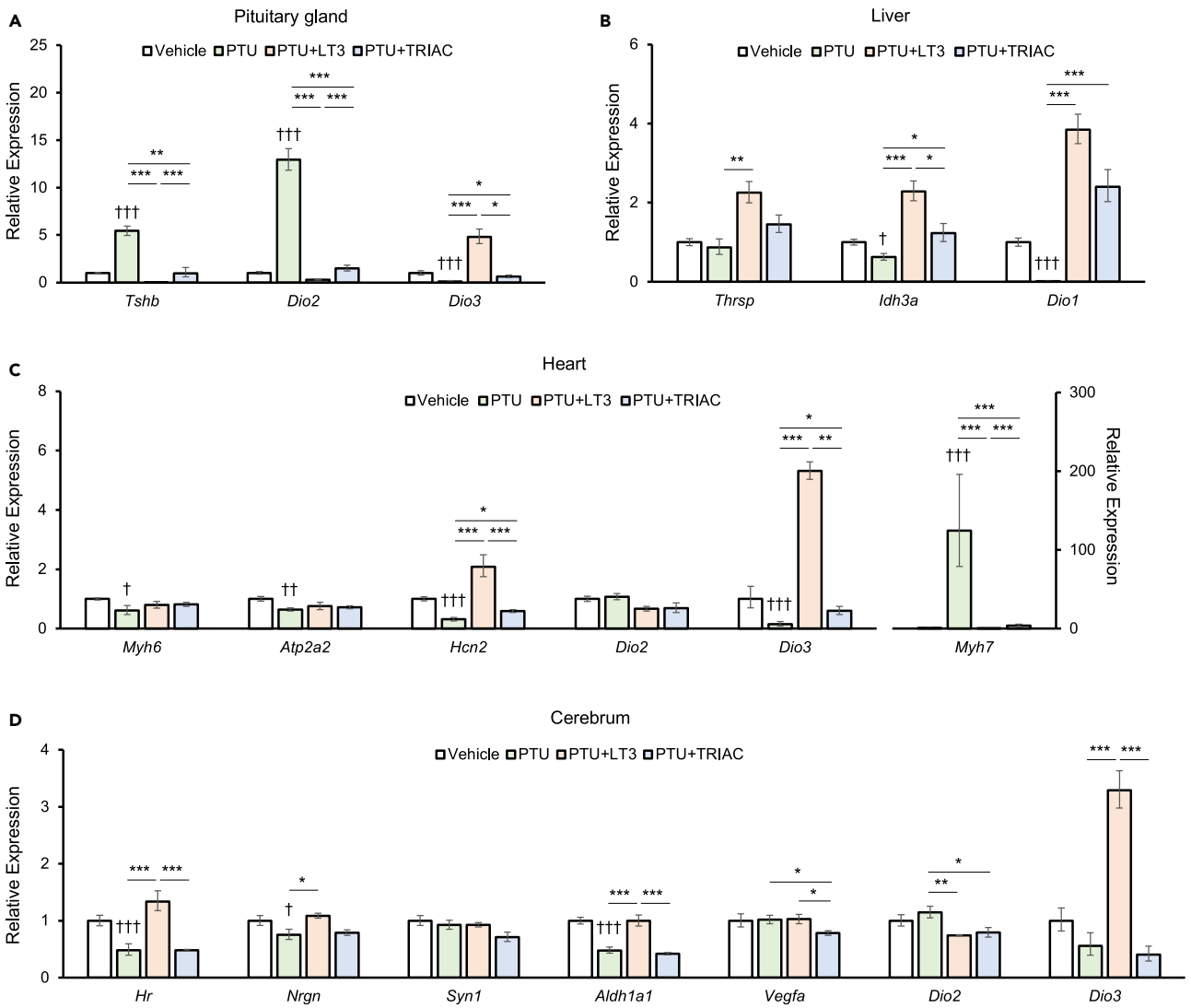


Figure 4. Gene expression profiles of hypothyroid mice determined by quantitative RT-PCR

(A) The pituitary gland, (B) the liver, (C) the heart, and (D) the cerebrum. Vehicle, PTU, PTU + TRIAC, n = 6 each; PTU + LT3, n = 5. Results are normalized using means of *Ppia* and *Hprt* as internal controls (see Table S3 for the individual results) and shown as fold change versus the vehicle-only control. Concentrations of PTU, LT3, and TRIAC used were 100 μg/mL, 0.1 μg/mL, and 0.1 μg/mL, respectively. Data are represented as means ± SEM. Statistical analyses were performed using Student's *t*-test for vehicle vs. PTU and the p value is presented as †p < 0.05, ††p < 0.01, and †††p < 0.001. ANOVA followed by the Tukey-Kramer test was used for comparisons among PTU, PTU + LT3, and PTU + TRIAC experiments and the p value is presented as *p < 0.05, **p < 0.01, and ***p < 0.001.

T4, LT3 co-administration increased T3, and TRIAC co-administration increased TRIAC as expected (Figures 5A–5C). By contrast, in the cerebrum, TRIAC co-administration did not increase TRIAC, whereas LT3 co-administration increased T3 (Figures 5D–5F). In summary, TRIAC administration does not increase cerebral TRIAC abundance, and TH-responsive genes therefore do not respond.

TRIAC administration to euthyroid mice

Based on the results of our experiments with hypothyroid mice, we hypothesized that exogenous TRIAC attenuates cerebral TH actions even in euthyroid mice. In detail, the supply of circulating T4 and T3 should be decreased by administering TRIAC, which suppresses the HPT axis (Figures 3D–3J). In addition, TRIAC does not appear to be transported into the cerebrum unlike T3 (Figures 5E and 5F). We therefore predicted that TRIAC administration cannot rescue the attenuation of cerebral TH actions due to a reduction in cerebral T4 and T3.

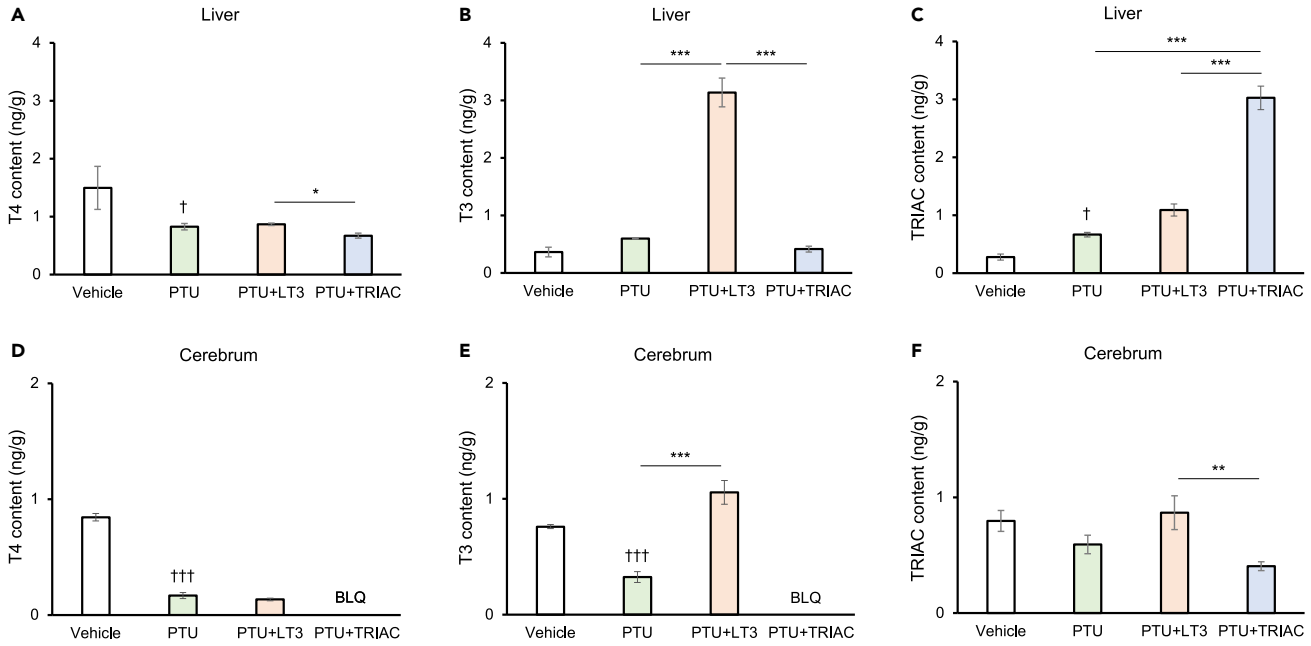


Figure 5. Organ-specific contents of thyroid hormones in hypothyroid mice

(A–C) Liver levels of T4 (A), T3 (B), and TRIAC (C).

(D–F) Cerebral levels of T4 (D), T3 (E), and TRIAC (F). Results are presented as amount of each thyroid hormone per gram of organ weight. Vehicle, PTU, PTU + TRIAC, n = 6 each; PTU + LT3, n = 5. Concentrations of PTU, LT3, and TRIAC used were 100 μg/mL, 0.1 μg/mL, and 0.1 μg/mL, respectively. Data are represented as means ± SEM. Statistical analyses were performed using Student's *t*-test for vehicle vs. PTU and the p value is presented as †$p < 0.05$ and †††$p < 0.001$. ANOVA followed by the Tukey-Kramer test was used for comparisons among PTU, PTU + LT3, and PTU + TRIAC experiments and the p value is presented as *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

To test our hypothesis, we further analyzed euthyroid mice with administering 0.1 μg/mL of either LT3 and TRIAC or 1 μg/mL (Figure 1). Amount of water consumption of each group is shown in Table S4: administration of either 1 μg/mL LT3 or 1 μg/mL TRIAC tended to increase water consumption. We found that serum TH levels changed as expected in these mice (Figures 6A–6C). Serum T4 levels were decreased by 0.1 μg/mL LT3 and by both 1 μg/mL LT3 and 1 μg/mL TRIAC (Figure 6A). Serum T3 levels were increased by 1 μg/mL LT3 but were decreased slightly by 0.1 μg/mL TRIAC and to a greater extent by 1 μg/mL TRIAC (Figure 6B). Serum TRIAC levels were increased by TRIAC administration in a dose-dependent manner and become slightly detectable by LT3 at 1 μg/mL (Figure 6C). We adopted 1 μg/mL as a working concentration because we verified substantial reduction in serum T4 and T3 levels following the administration of 1 μg/mL TRIAC.

We subsequently measured gene expression in the liver and cerebrum. LT3 administration increased the abundance of TH-responsive gene transcripts in both the liver and cerebrum (Figures 6D and 6E). Similarly to hypothyroid mice (Figure 4D), the abundance of *Dio2* mRNA in the cerebrum decreased while that of *Dio3* mRNA increased following 1 μg/mL LT3 administration (Figure 6E). Meanwhile, TRIAC administration did not increase the abundance of TH-responsive gene transcripts in the cerebrum. Instead, *Hr* and *Vegfa* mRNA levels were lower even though the mRNAs of TH-responsive genes were significantly higher in the liver (Figures 6F and 6G). Furthermore, cerebral *Dio3* mRNA levels were unchanged following TRIAC administration (Figure 6G). We verified consistency of individual results by normalizing against *Ppia* and *Hprt* (Table S5). Similar results were obtained by normalizing against *Gapdh* and *Actb* as other internal controls, which supported relevance of *Ppia* and *Hprt* use (Figure S3).

We proceeded to measure organ-specific TH contents to determine whether quantitative changes in local THs may be responsible for the observed downregulation of cerebral TH-responsive genes in response to TRIAC. Similarly to serum levels (Figures 6A–6C), liver T3 and T4 contents were both altered by LT3 and by TRIAC administration (Figures 7A–7C). Liver T4 contents were decreased by both 1 μg/mL LT3 and 1 μg/mL TRIAC (Figure 7A). Liver T3 contents were increased by 1 μg/mL LT3 but unchanged by 1 μg/mL TRIAC

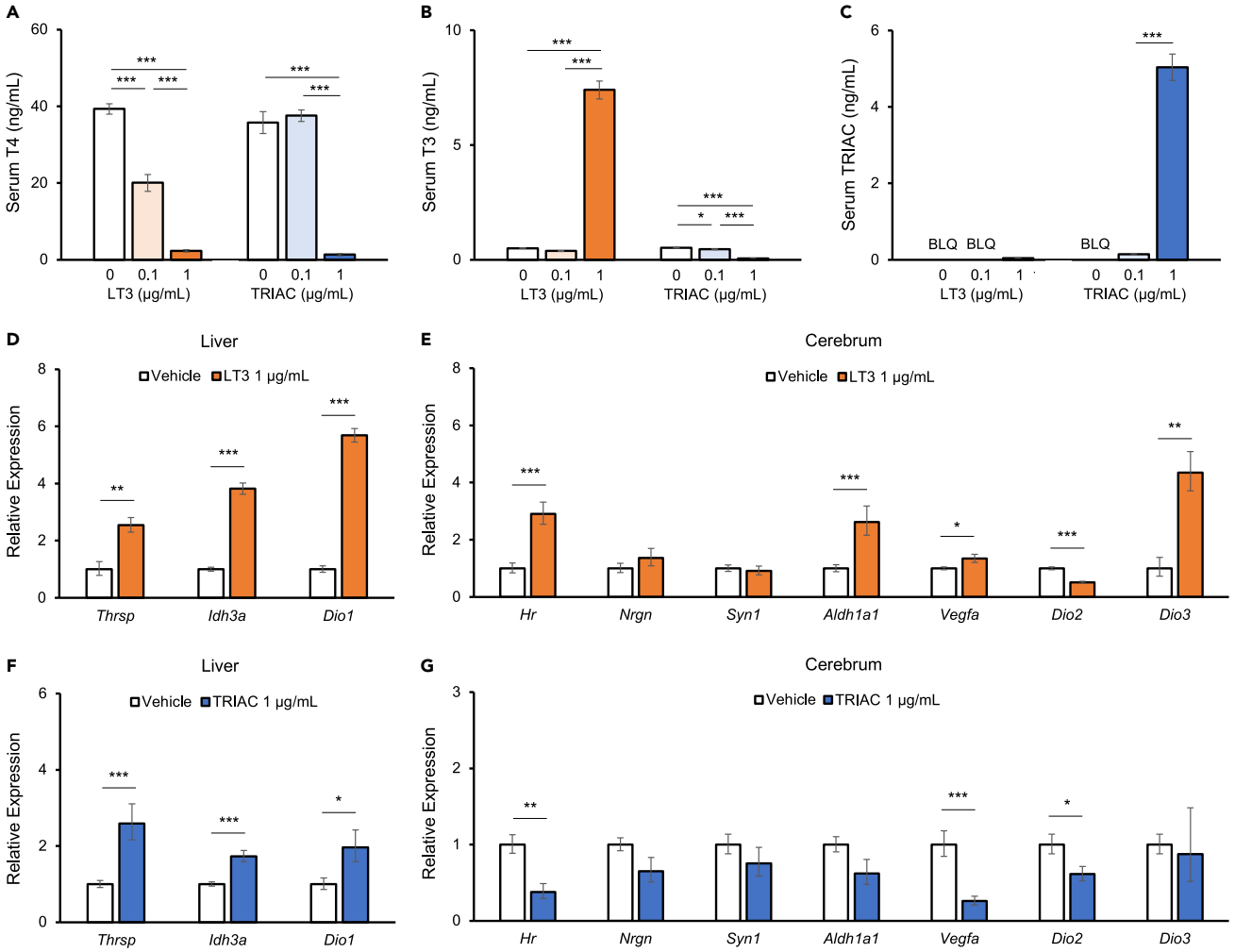


Figure 6. Serum thyroid hormone levels and gene expression profiles of euthyroid mice

(A–C) Serum levels of T4 (A), T3 (B), and TRIAC (C) in mice administered with 0.1 μg/mL of either LT3 and TRIAC or 1 μg/mL (n = 6 each).

(D–F) Gene expression profiles determined by quantitative RT-PCR; liver (D) and cerebrum (E) of mice administered with 1 μg/mL LT3 (n = 6 each); liver (F) and cerebrum (G) of mice administered with 1 μg/mL TRIAC (n = 6 each). Results are normalized using means of *Ppia* and *Hprt* as internal controls (see Table S5 for the individual results) and shown as fold change versus the vehicle-only control. Data are represented as means ± SEM. Statistical analyses were performed by ANOVA followed by the Tukey-Kramer test for panels A–C and by Student's *t*-test for panels D-G. *p < 0.05, **p < 0.01, and ***p < 0.001. See also Figures S3, S5, and Table S4.

(Figure 7B). Liver TRIAC contents were increased by TRIAC administration in a dose-dependent manner but unchanged by LT3 (Figure 7C). We also detected changes in cerebral T3 and T4 contents. LT3 administration decreased cerebral T4 contents, increased T3 contents, and decreased TRIAC contents (Figures 7D–7F). By contrast, cerebral T4 and T3 contents decreased following TRIAC administration while TRIAC contents remained unchanged (Figures 7D–7F). We additionally analyzed perfused livers to evaluate without blood contamination. As a result, T4 and TRIAC contents of perfused livers were changed by TRIAC administration in a similar manner to non-perfused livers (Figure S4).

We further examined elements that regulate TH actions to verify that the HPT axis mediates changes in overall and organ-specific levels of THs following TRIAC administration. We observed significant changes in liver mRNA levels of *Thra* and *Thrb*, which encode THRs, as well as *Mct8*, a TH transporter. The levels of these three transcripts were higher following LT3 administration but lower after TRIAC administration (Figures S5A and S5B). Nevertheless, TRIAC upregulated liver TH-responsive genes (Figure 6F). As for genes that mediate the glucuronidation of THs, *Ugt1a1* mRNA was increased by LT3 but not significantly

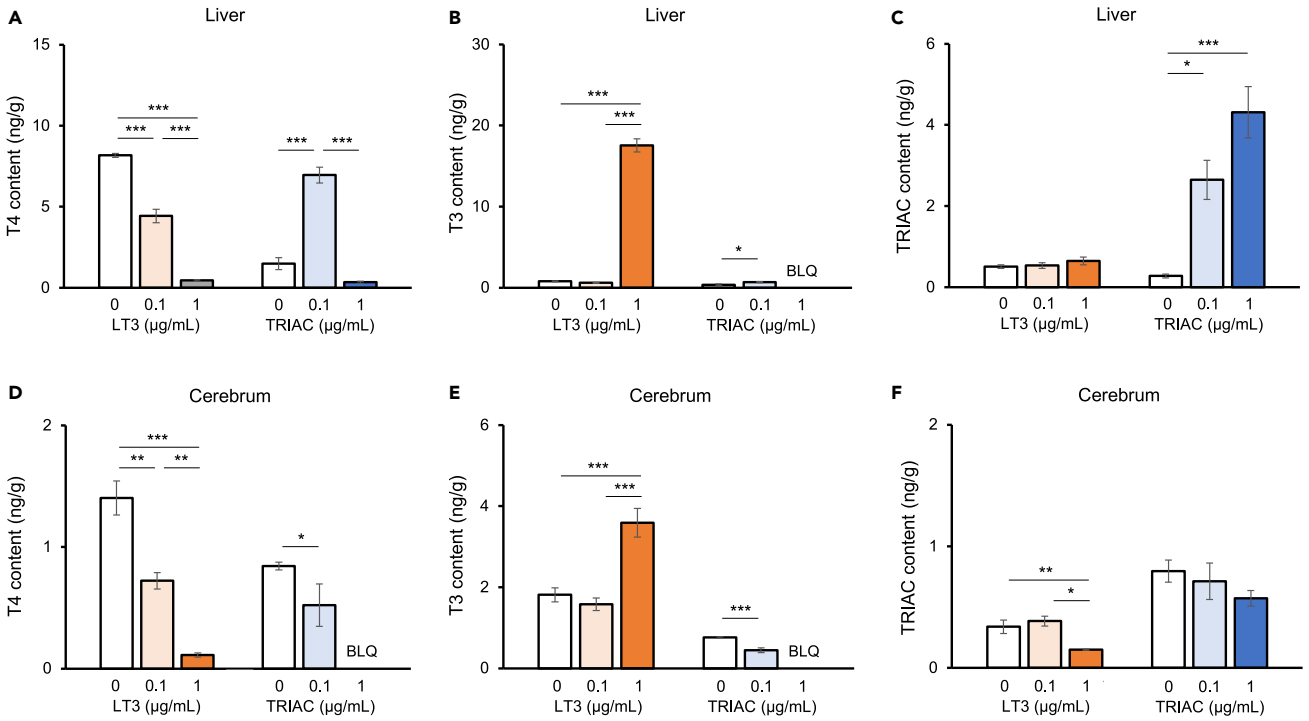


Figure 7. Organ-specific contents of thyroid hormones in euthyroid mice

(A–C) Liver-specific levels of T4 (A), T3 (B), and TRIAC (C) in mice administered with 0.1 μg/mL of either LT3 and TRIAC or 1 μg/mL (n = 6 each).
(D–F) Cerebral levels of T4 (D), T3 (E), and TRIAC (F) in mice administered with 0.1 μg/mL of either LT3 and TRIAC or 1 μg/mL (n = 6 each). Results are presented as amount of each thyroid hormone per gram of tissue weight. Data are represented as means ± SEM. Statistical analyses all involved ANOVA followed by the Tukey-Kramer test. *p < 0.05, **p < 0.01, and ***p < 0.001. See also Figures S4 and S5.

changed by TRIAC, whereas *Ugt1a9* mRNA was decreased by both LT3 and TRIAC (Figures S5A and S5B). The serum TH-lowering effects of TRIAC are apparently not due to glucuronidation in the liver. The significant changes observed in the cerebrum were a decrease in *Thra* mRNA following LT3 administration and a decrease in *Oatp1c1* mRNA, another TH transporter, following TRIAC administration (Figures S5C and S5D). However, the result of *Oatp1c1* was inconclusive: the difference was not significant when normalized by *Ppia* (Table S6). Cerebral *Mct8* mRNA did not change by either LT3 or TRIAC.

As for the pituitary gland, mRNA levels of *Tshb* and *Dio2* were decreased and *Dio3* mRNA was increased by administering either 1 μg/mL LT3 or 1 μg/mL TRIAC to euthyroid mice (Figure 8A). We verified consistency of individual results by normalizing against *Ppia* and *Hprt* (Table S7). Moreover, administration of either LT3 or TRIAC reduced thyroid gland size (Figures 8B and 8C) and thickness of follicular cells (Figures 8D–8F). On the other hand, whether lower concentration of TRIAC could affect HPT axis was examined. Administration of 0.01 μg/mL TRIAC to hypothyroid mice significantly reduced serum TSH levels (Figure 8G) and thyroid gland size (Figures 8H–8J). Amount of water consumption was similar between each group (Table S8).

DISCUSSION

As environmental water could be contaminated with TRIAC,[18] we determined the biological effects of exogenous TRIAC. Comparisons were performed against T3, a main endogenous contributor of TH actions. By administering either TRIAC or T3 to hypothyroid mice, we found both compounds exerted similar effects on growth and the HPT axis. Specifically, TRIAC administration upregulated TH-responsive genes of the pituitary gland, the liver, and the heart, as did T3. Interestingly, we observed that TRIAC administration could not rescue the downregulation of TH-responsive genes in the cerebrums of hypothyroid mice. By measuring organ-specific TH contents, we found that TRIAC was not sufficiently distributed into the cerebrum when administered orally. Altogether, we verified that TRIAC administration attenuates cerebral TH actions even in the euthyroid state. The mechanism underlying TH depletion in the cerebrum appears to involve suppression of the HPT axis and disabling TRIAC delivery to the cerebrum.

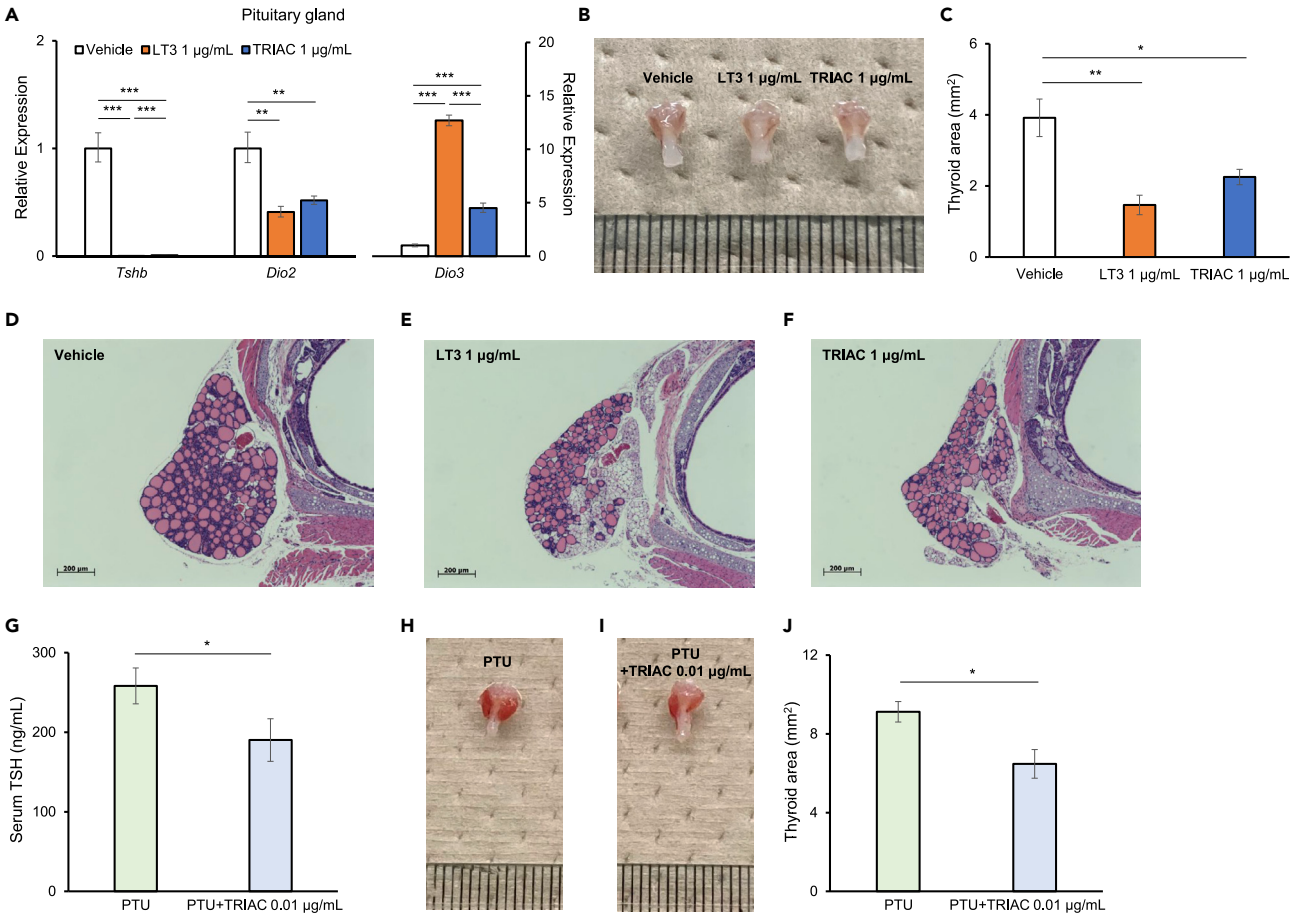


Figure 8. TRIAC effects on the hypothalamus-pituitary-thyroid axis

(A–F) Results of euthyroid mice administered with 1 μg/mL LT3 (n = 4 each) and those with 1 μg/mL TRIAC (n = 4 each).

(A) Gene expression profiles of the pituitary gland were determined by quantitative RT-PCR. Results are normalized using means of *Ppia* and *Hprt* as internal controls (see Table S7 for the individual results).

(B) Gross appearance of thyroid glands. The scale on the ruler is 1 mm.

(C) Thyroid gland size was measured as thyroid area on photograph.

(D–F) Histological images of thyroid glands stained using hematoxylin and eosin.

(G–J) Results of hypothyroid mice administered with 0.01 μg/mL TRIAC (n = 4 each); serum TSH levels (G), gross appearance of thyroid glands (H, I), and thyroid gland size (J). The scale on the ruler is 1 mm. Concentrations of PTU used were 100 μg/mL. Data are represented as means ± SEM. Statistical analyses were performed by ANOVA followed by the Tukey-Kramer test for panels A–C and by Student's *t*-test for panels G and H. *p < 0.05, **p < 0.01, and ***p < 0.001. See also Table S8.

Euthyroid models have conventionally been used to evaluate EDCs.[1,28] In this study, we induced hypothyroidism in mice using PTU to investigate effects on TH action with high sensitivity. Growth effects could not be determined in euthyroid mice but could be determined in hypothyroid mice as growth retardation was restored by LT3 and TRIAC administration. Moreover, hypothyroid mice visualized changes in the HPT axis as enlargement of the thyroid gland and follicular dysmorphology. This observation reflects the stimulating effects of elevated circulating TSH via feedback reaction against TH depletion. In addition, hypothyroid mice enabled changes in TH-responsive gene expression to be measured clearly. We administered 0.1 μg/mL TRIAC to hypothyroid mice, which led to 20 μg/kg/day of TRIAC intake. This dose was in the lower range of the doses used in previous animal studies summarized in this review.[7] We believe that other EDCs and candidates can be further elucidated using hypothyroid mice.

We could interpret TH-responsive gene profiles using the results of controls, namely PTU administration for downregulation and LT3 co-administration for upregulation. We verified the upregulatory effects of TRIAC on multiple TH-responsive genes in the pituitary gland, the liver, and the heart. In the cerebrum, *Hr* and

Aldh1a1 mRNA levels were decreased by PTU administration and this decrease was rescued by LT3 co-administration. However, TRIAC co-administration could not restore the downregulation of *Hr* and *Aldh1a1*. We observed a similar discrepancy for *Dio3*; both LT3 and TRIAC co-administration upregulated *Dio3* mRNA levels in the heart, whereas only LT3 upregulated *Dio3* in the cerebrum. DIO3 decreases cellular T3 by 5′-deiodination and its activity in the cerebral cortex is increased in hyperthyroid rats and decreased in hypothyroid rats.[29]

Gene expression profiles of hypothyroid mice indicated that exogenous TRIAC does not augment TH actions in the cerebrum. The underlying mechanism was elucidated by comparing the TH contents of the liver and the cerebrum. Liver T3 and TRIAC contents increased following administration of the respective compound. By contrast, cerebral TRIAC contents were not increased by its administration although cerebral T3 levels were increased by LT3 administration. Thus, TRIAC does not appear to be trafficked into the cerebrum, which could explain why cerebral TH-responsive genes are unresponsive to exogenous TRIAC.

T4 and T3, major endogenous THR agonists, cross plasma membranes via transporters such as the monocarboxylate transporters (MCTs), organic anion-transporting polypeptides (OATPs), L-type amino acid transporters, and sodium/taurocholate cotransporting polypeptide.[30] MCT8 is an essential transporter of T4 and T3 particularly in central nervous system (CNS). Patients with mutations in *MCT8* develop Allan-Herndon-Dudley syndrome, which is characterized by a severe neurodevelopmental defect, as well as thyroid dysfunction.[31] *Mct8*-knockout mice have less T4 and T3 in their brains compared with wild type.[32] Meanwhile, OATP1C1 can transport T4 into the brain because brain T4 levels are even lower in *Mct8*/*Oatp1c1* double knockout mice compared with *Mct8*-knockout mice.[33] However, OATP1C1 concentration within the human blood-brain barrier (BBB) is much lower than in the murine BBB.[34]

The potential to be transported by either MCT8, OATP1C1, or both is quite important for TH action in CNS of EDCs because MCT8 and OATP1C1 are both expressed in the human BBB.[34] Importantly, TRIAC is apparently not transported by MCT8.[35] Although there have been no reports on the relationship between TRIAC and OATP1C1, TETRAC, a precursor hormone of TRIAC, is not transported by either MCT8 or OATP1C1.[36] In this context, insufficient CNS distribution of exogenous TRIAC was assumed to be due to poor permeability through the BBB. This assumption was supported not only by our results but also by other reports that TRIAC administration does not upregulate TH-responsive genes in the mouse brain.[37,38]

The results from hypothyroid mice raised the concern that exogenous intake of TRIAC can disrupt TH actions in the CNS. First, exogenous TRIAC decreases TH secretion from the thyroid gland by suppressing the HPT axis. Second, the decrease in circulating TH levels caused by TRIAC leads to insufficient TH levels and attenuation of TH actions in various tissues. Third, exogenous TRIAC compensates for TH actions by acting as an alternative to endogenous THs in peripheral tissues. However, TRIAC does not compensate for TH actions in the CNS because TRIAC hardly distributes through the BBB. We tested our hypothesis by analyzing euthyroid mice administered TRIAC. TRIAC administration decreased serum levels and the liver contents of T4 and T3 but transcript levels of TH-responsive genes were increased in the liver. Meanwhile, TRIAC administration failed to increase TRIAC contents and instead, downregulated TH-responsive genes in the cerebrum. In other words, TRIAC administration depleted T4 and T3 from the cerebrum without TRIAC being trafficked into the cerebrum, which resulted in the downregulation of TH-responsive genes in the cerebrum. Additionally, we determined that the HPT axis is the main contributor to the depletion of THs by TRIAC administration and that the expression of genes encoding THRs and transporters did not change significantly in the cerebrum.

The mechanisms of EDC action have been proposed in an expert consensus statement.[8] Of the characteristics presented in the statement, our observations suggest that TRIAC exerts its disruptive effects by activating hormone receptors, interfering with hormone synthesis, and altering circulating hormone levels. We also determined that these multiple characteristics contribute cooperatively to the disruptive effects of TRIAC. Furthermore, the key disruptive characteristic of TRIAC is its heterogeneous distribution among organs, specifically its poor permeability across the BBB. We have therefore identified a curious concept of EDC action.

As described in the introduction, the effluent from several sewage treatment plants have been found to contain TRIAC, which suggests that environmental water can be contaminated by TRIAC.[18] In wildlife, malformations were developed not only by T4 and T3 but by exposure to TRIAC.[39] The data we have presented

here provides a warning of an adverse risk posed by environmental TRIAC toward the human CNS. THs play crucial roles in fetal and neonatal neurodevelopment because profound and permanent CNS defects have been observed in patients with untreated congenital hypothyroidism, resistance to thyroid hormone α,[40,41] and Allan-Herndon-Dudley syndrome.[31] Even milder attenuation of TH actions in the CNS could pose a significant risk to neurodevelopment. For example, children with permanent congenital hypothyroidism were reported to have lower cognitive scores than those with transient congenital hypothyroidism and control subjects even if they received replacement therapy.[42]

In clinical settings, TRIAC is being developed as a therapeutic agent for Allan-Herndon-Dudley syndrome.[43,44] TRIAC treatment ameliorated high serum T3 levels, cardiovascular status, and hypermetabolic state in peripheral tissues.[43] An international phase IIb clinical trial is ongoing (NCT02396459) because TRIAC effects on neurodevelopmental outcomes were not significantly seen so far.[43,44] With regard to this controversy, Bárez-López et al. reported that TRIAC administration decreased cerebral T3 contents as well as plasma T4 levels, which was determined by 9 days of administration from postnatal day 21 to wild type and *Mct8*-knockout mice with C57BL/6J background.[37] Here, we further confirmed that wild-type mice occurred brain hypothyroidism by TRIAC administration. The caution for clinical use of TRIAC from the perspective of neurodevelopment seemed to be given to Allan-Herndon-Dudley syndrome as well as resistance to thyroid hormone β.

The measurement of TRIAC in various settings is important for further investigation. TRIAC levels in umbilical cord blood may directly suggest a neurodevelopment risk. Similarly, serum TRIAC levels or the ratio of TRIAC to other THs may be a biomarker for cognitive risk both in children and adults. The impact of long-term low-dose exposure can be evaluated by an ongoing birth cohort study with environmental TRIAC monitoring.

In conclusion, exogenous TRIAC intake can attenuate TH actions exclusively in the cerebrum. The disruptive mechanisms of TRIAC were characterized as the additive effect of circulating endogenous THs being depleted via negative feedback acting on the HPT axis, THR activation in peripheral tissues, and poor permeability across the BBB. Our finding gives caution for exogenous TRIAC and offers epidemiological studies in combination with TRIAC measurement.

Limitation of the study

The present study has a limitation that significant effects of TRIAC at an environmental concentration have not been determined. In our previous report, the highest concentration of TRIAC detected in sewage effluents was 4.2 ng/L.[18] To the best of our ability, we could confirm TRIAC effects at as low as 0.01 μg/mL that was still higher than the environmental concentration, even if we took human equivalent dose into consideration.[45] To investigate with high sensitivity, cohort studies including TRIAC measurement may be rather promising as a large number of subjects can be recruited.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
  - Animals
- METHOD DETAILS
  - Chemical administration to mice and sample collection
  - Thyroid hormone measurement of serum
  - Thyroid hormone measurement of organ
  - RNA extraction and quantitative RT-PCR
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.107135.

ACKNOWLEDGMENTS

This work was supported by the Environment Research and Technology Development Fund (JPMEERF20195053, JPMEERF20235003) of the Environmental Restoration and Conservation Agency of Japan.

AUTHOR CONTRIBUTIONS

Conceptualization, I.Y.; experiments using mice and analyses without LC-MS/MS, I.Y., T.H., Y.U., and T.S.; analyses with LC-MS/MS, R.O., Y.T., M.Y., S.F.N., and D.N.; funding acquisition, T.K.; supervision, N.I. I.Y. drafted the manuscript, and all authors reviewed, edited, and approved the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing financial interests.

Received: December 20, 2022
Revised: April 25, 2023
Accepted: June 12, 2023
Published: June 15, 2023

REFERENCES

1. Gore, A.C., Chappell, V.A., Fenton, S.E., Flaws, J.A., Nadal, A., Prins, G.S., Toppari, J., and Zoeller, R.T. (2015). EDC-2: the endocrine society's second scientific statement on endocrine-disrupting chemicals. Endocr. Rev. *36*, E1–E150. https://doi.org/10.1210/er.2015-1010.

2. Maia, A.L., Kim, B.W., Huang, S.A., Harney, J.W., and Larsen, P.R. (2005). Type 2 iodothyronine deiodinase is the major source of plasma T3 in euthyroid humans. J. Clin. Invest. *115*, 2524–2533. https://doi.org/10.1172/jci25083.

3. Jongejan, R.M.S., Meima, M.E., Visser, W.E., Korevaar, T.I.M., van den Berg, S.A.A., Peeters, R.P., and de Rijke, Y.B. (2022). Binding characteristics of thyroid hormone distributor proteins to thyroid hormone metabolites. Thyroid *32*, 990–999. https://doi.org/10.1089/thy.2021.0588.

4. Schueler, P.A., Schwartz, H.L., Strait, K.A., Mariash, C.N., and Oppenheimer, J.H. (1990). Binding of 3,5,3′-triiodothyronine (T3) and its analogs to the in vitro translational products of c-erbA protooncogenes: differences in the affinity of the alpha- and beta-forms for the acetic acid analog and failure of the human testis and kidney alpha-2 products to bind T3. Mol. Endocrinol. *4*, 227–234. https://doi.org/10.1210/mend-4-2-227.

5. Takeda, T., Suzuki, S., Liu, R.T., and DeGroot, L.J. (1995). Triiodothyroacetic acid has unique potential for therapy of resistance to thyroid hormone. J. Clin. Endocrinol. Metab. *80*, 2033–2040. https://doi.org/10.1210/jcem.80.7.7608251.

6. Martínez, L., Nascimento, A.S., Nunes, F.M., Phillips, K., Aparicio, R., Dias, S.M.G., Figueira, A.C.M., Lin, J.H., Nguyen, P., Apriletti, J.W., et al. (2009). Gaining ligand selectivity in thyroid hormone receptors via entropy. Proc. Natl. Acad. Sci. USA *106*, 20717–20722. https://doi.org/10.1073/pnas.0911024106.

7. Groeneweg, S., Peeters, R.P., Visser, T.J., and Visser, W.E. (2017). Triiodothyroacetic acid in health and disease. J. Endocrinol. *234*, R99–R121. https://doi.org/10.1530/joe-17-0113.

8. La Merrill, M.A., Vandenberg, L.N., Smith, M.T., Goodson, W., Browne, P., Patisaul, H.B., Guyton, K.Z., Kortenkamp, A., Cogliano, V.J., Woodruff, T.J., et al. (2020). Consensus on the key characteristics of endocrine-disrupting chemicals as a basis for hazard identification. Nat. Rev. Endocrinol. *16*, 45–57. https://doi.org/10.1038/s41574-019-0273-8.

9. Wild, C.P. (2005). Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. Cancer Epidemiol. Biomarkers Prev. *14*, 1847–1850. https://doi.org/10.1158/1055-9965.epi-05-0456.

10. Moriyama, K., Tagami, T., Akamizu, T., Usui, T., Saijo, M., Kanamoto, N., Hataya, Y., Shimatsu, A., Kuzuya, H., and Nakao, K. (2002). Thyroid hormone action is disrupted by bisphenol A as an antagonist. J. Clin. Endocrinol. Metab. *87*, 5185–5190. https://doi.org/10.1210/jc.2002-020209.

11. Dong, H., and Wade, M.G. (2017). Application of a nonradioactive assay for high throughput screening for inhibition of thyroid hormone uptake via the transmembrane transporter MCT8. Toxicol. Vitro *40*, 234–242. https://doi.org/10.1016/j.tiv.2017.01.014.

12. Xu, X., Fan, S., Guo, Y., Tan, R., Zhang, J., Zhang, W., Pan, B.X., and Kato, N. (2019). The effects of perinatal bisphenol A exposure on thyroid hormone homeostasis and glucose metabolism in the prefrontal cortex and hippocampus of rats. Brain Behav. *9*, e01225. https://doi.org/10.1002/brb3.1225.

13. Greer, M.A., Goodman, G., Pleus, R.C., and Greer, S.E. (2002). Health effects assessment for environmental perchlorate contamination: the dose response for inhibition of thyroidal radioiodine uptake in humans. Environ. Health Perspect. *110*, 927–937. https://doi.org/10.1289/ehp.02110927.

14. Murata, T., and Yamauchi, K. (2008). 3,3′,5-Triiodo-L-thyronine-like activity in effluents from domestic sewage treatment plants detected by in vitro and in vivo bioassays. Toxicol. Appl. Pharmacol. *226*, 309–317. https://doi.org/10.1016/j.taap.2007.09.003.

15. Jugan, M.L., Oziol, L., Bimbot, M., Huteau, V., Tamisier-Karolak, S., Blondeau, J.P., and Lévi, Y. (2009). In vitro assessment of thyroid and estrogenic endocrine disruptors in wastewater treatment plants, rivers and drinking water supplies in the greater Paris area (France). Sci. Total Environ. *407*, 3579–3587. https://doi.org/10.1016/j.scitotenv.2009.01.027.

16. Kusk, K.O., Krüger, T., Long, M., Taxvig, C., Lykkesfeldt, A.E., Frederiksen, H., Andersson, A.M., Andersen, H.R., Hansen, K.M.S., Nellemann, C., et al. (2011). Endocrine potency of wastewater: contents of endocrine disrupting chemicals and effects measured by in vivo and in vitro assays. Environ. Toxicol. Chem. *30*, 413–426. https://doi.org/10.1002/etc.385.

17. Kassotis, C.D., Iwanowicz, L.R., Akob, D.M., Cozzarelli, I.M., Mumford, A.C., Orem, W.H., and Nagel, S.C. (2016). Endocrine disrupting activities of surface water associated with a West Virginia oil and gas industry wastewater disposal site. Sci. Total Environ. *557–558*, 901–910. https://doi.org/10.1016/j.scitotenv.2016.03.113.

18. Omagari, R., Yagishita, M., Shiraishi, F., Nakayama, S.F., Terasaki, M., Tanigawa, T., Yamauchi, I., Kubo, T., and Nakajima, D. (2022). Identification by liquid chromatography-tandem mass spectrometry and liquid chromatography-quadrupole time-of-flight mass spectrometry of the contributor to the thyroid hormone receptor agonist activity in effluents from sewage treatment plants. Environ. Sci. Technol. *56*,

13709–13718. https://doi.org/10.1021/acs.est.2c02648.

19. Yamauchi, I., Sakane, Y., Yamashita, T., Hakata, T., Sugawa, T., Fujita, H., Okamoto, K., Taura, D., Hirota, K., Ueda, Y., et al. (2022). Thyroid hormone economy in mice overexpressing iodothyronine deiodinases. FASEB J. *36*, e22141. https://doi.org/10.1096/fj.202101288RR.

20. Yamauchi, I., Yamashita, T., Sugawa, T., Tagami, T., Hanaoka, I., Usui, T., Hirota, K., Hakata, T., Ueda, Y., Fujii, T., et al. (2022). Bezafibrate induces hypothyroidism in a patient with resistance to thyroid hormone β due to a G347R variant. Clin. Endocrinol. *96*, 236–245. https://doi.org/10.1111/cen.14591.

21. Yamauchi, I., Sakane, Y., Okuno, Y., Sugawa, T., Hakata, T., Fujita, H., Okamoto, K., Taura, D., Yamashita, T., Hirota, K., et al. (2022). High-throughput screening in combination with a cohort study for iodothyronine deiodinases. Endocrinology *163*, bqac090. https://doi.org/10.1210/endocr/bqac090.

22. Kaneshige, M., Kaneshige, K., Zhu, X., Dace, A., Garrett, L., Carter, T.A., Kazlauskaite, R., Pankratz, D.G., Wynshaw-Boris, A., Refetoff, S., et al. (2000). Mice with a targeted mutation in the thyroid hormone beta receptor gene exhibit impaired growth and resistance to thyroid hormone. Proc. Natl. Acad. Sci. USA *97*, 13209–13214. https://doi.org/10.1073/pnas.230285997.

23. Yamashita, T., Fujii, T., Yamauchi, I., Ueda, Y., Hirota, K., Kanai, Y., Yasoda, A., and Inagaki, N. (2020). C-Type natriuretic peptide restores growth impairment under enzyme replacement in mice with mucopolysaccharidosis VII. Endocrinology *161*, bqaa008. https://doi.org/10.1210/endocr/bqaa008.

24. Bianco, A.C., Anderson, G., Forrest, D., Galton, V.A., Gereben, B., Kim, B.W., Kopp, P.A., Liao, X.H., Obregon, M.J., Peeters, R.P., et al. (2014). American Thyroid Association Guide to investigating thyroid hormone economy and action in rodent and cell models. Thyroid *24*, 88–168. https://doi.org/10.1089/thy.2013.0109.

25. Menegay, C., Juge, C., and Burger, A.G. (1989). Pharmacokinetics of 3,5,3′-triiodothyroacetic acid and its effects on serum TSH levels. Acta Endocrinol. *121*, 651–658. https://doi.org/10.1530/acta.0.1210651.

26. Goslings, B., Schwartz, H.L., Dillmann, W., Surks, M.I., and Oppenheimer, J.H. (1976). Comparison of the metabolism and distribution of L-triiodothyronine and triiodothyroacetic acid in the rat: a possible explanation of differential hormonal potency. Endocrinology *98*, 666–675. https://doi.org/10.1210/endo-98-3-666.

27. Bianco, A.C., Salvatore, D., Gereben, B., Berry, M.J., and Larsen, P.R. (2002). Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocr. Rev. *23*, 38–89. https://doi.org/10.1210/edrv.23.1.0455.

28. Lu, L., Wu, H., Cui, S., Zhan, T., Zhang, C., Lu, S., Liu, W., and Zhuang, S. (2020). Pentabromoethylbenzene exposure induces transcriptome aberration and thyroid dysfunction: in vitro, in silico, and in vivo investigations. Environ. Sci. Technol. *54*, 12335–12344. https://doi.org/10.1021/acs.est.0c03308.

29. Kaplan, M.M., and Yaskoski, K.A. (1980). Phenolic and tyrosyl ring deiodination of iodothyronines in rat brain homogenates. J. Clin. Invest. *66*, 551–562. https://doi.org/10.1172/jci109887.

30. Visser, W.E., Friesema, E.C.H., and Visser, T.J. (2011). Minireview: thyroid hormone transporters: the knowns and the unknowns. Mol. Endocrinol. *25*, 1–14. https://doi.org/10.1210/me.2010-0095.

31. Friesema, E.C.H., Grueters, A., Biebermann, H., Krude, H., von Moers, A., Reeser, M., Barrett, T.G., Mancilla, E.E., Svensson, J., Kester, M.H.A., et al. (2004). Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. Lancet *364*, 1435–1437. https://doi.org/10.1016/s0140-6736(04)17226-7.

32. Trajkovic, M., Visser, T.J., Mittag, J., Horn, S., Lukas, J., Darras, V.M., Raivich, G., Bauer, K., and Heuer, H. (2007). Abnormal thyroid hormone metabolism in mice lacking the monocarboxylate transporter 8. J. Clin. Invest. *117*, 627–635. https://doi.org/10.1172/jci28253.

33. Mayerl, S., Müller, J., Bauer, R., Richert, S., Kassmann, C.M., Darras, V.M., Buder, K., Boelen, A., Visser, T.J., and Heuer, H. (2014). Transporters MCT8 and OATP1C1 maintain murine brain thyroid hormone homeostasis. J. Clin. Invest. *124*, 1987–1999. https://doi.org/10.1172/jci70324.

34. Roberts, L.M., Woodford, K., Zhou, M., Black, D.S., Haggerty, J.E., Tate, E.H., Grindstaff, K.K., Mengesha, W., Raman, C., and Zerangue, N. (2008). Expression of the thyroid hormone transporters monocarboxylate transporter-8 (SLC16A2) and organic ion transporter-14 (SLCO1C1) at the blood-brain barrier. Endocrinology *149*, 6251–6261. https://doi.org/10.1210/en.2008-0378.

35. Kersseboom, S., Horn, S., Visser, W.E., Chen, J., Friesema, E.C.H., Vaurs-Barrière, C., Peeters, R.P., Heuer, H., and Visser, T.J. (2014). In vitro and mouse studies supporting therapeutic utility of triiodothyroacetic acid in MCT8 deficiency. Mol. Endocrinol. *28*, 1961–1970. https://doi.org/10.1210/me.2014-1135.

36. Horn, S., Kersseboom, S., Mayerl, S., Müller, J., Groba, C., Trajkovic-Arsic, M., Ackermann, T., Visser, T.J., and Heuer, H. (2013). Tetrac can replace thyroid hormone during brain development in mouse mutants deficient in the thyroid hormone transporter mct8. Endocrinology *154*, 968–979. https://doi.org/10.1210/en.2012-1628.

37. Bárez-López, S., Obregon, M.J., Martínez-de-Mena, R., Bernal, J., Guadaño-Ferraz, A., and Morte, B. (2016). Effect of triiodothyroacetic acid treatment in Mct8 deficiency: a word of caution. Thyroid *26*, 618–626. https://doi.org/10.1089/thy.2015.0388.

38. Bárez-López, S., Grijota-Martínez, C., Liao, X.H., Refetoff, S., and Guadaño-Ferraz, A. (2019). Intracerebroventricular administration of the thyroid hormone analog TRIAC increases its brain content in the absence of MCT8. PLoS One *14*, e0226017. https://doi.org/10.1371/journal.pone.0226017.

39. Haggard, D.E., Noyes, P.D., Waters, K.M., and Tanguay, R.L. (2018). Transcriptomic and phenotypic profiling in developing zebrafish exposed to thyroid hormone receptor agonists. Reprod. Toxicol. *77*, 80–93. https://doi.org/10.1016/j.reprotox.2018.02.006.

40. Bochukova, E., Schoenmakers, N., Agostini, M., Schoenmakers, E., Rajanayagam, O., Keogh, J.M., Henning, E., Reinemund, J., Gevers, E., Sarri, M., et al. (2012). A mutation in the thyroid hormone receptor alpha gene. N. Engl. J. Med. *366*, 243–249. https://doi.org/10.1056/NEJMoa1110296.

41. Rurale, G., Di Cicco, E., Dentice, M., Salvatore, D., Persani, L., Marelli, F., and Luongo, C. (2019). Thyroid hormone hyposensitivity: from genotype to phenotype and back. Front. Endocrinol. *10*, 912. https://doi.org/10.3389/fendo.2019.00912.

42. Perri, K., De Mori, L., Tortora, D., Calevo, M.G., Allegri, A.E.M., Napoli, F., Patti, G., Fava, D., Crocco, M., Schiavone, M., et al. (2021). Cognitive and white matter microstructure development in congenital hypothyroidism and familial thyroid disorders. J. Clin. Endocrinol. Metab. *106*, e3990–e4006. https://doi.org/10.1210/clinem/dgab412.

43. Groeneweg, S., Peeters, R.P., Moran, C., Stoupa, A., Auriol, F., Tonduti, D., Dica, A., Paone, L., Rozenkova, K., Malikova, J., et al. (2019). Effectiveness and safety of the tri-iodothyronine analogue Triac in children and adults with MCT8 deficiency: an international, single-arm, open-label, phase 2 trial. Lancet Diabetes Endocrinol. *7*, 695–706. https://doi.org/10.1016/s2213-8587(19)30155-x.

44. van Geest, F.S., Groeneweg, S., van den Akker, E.L.T., Bacos, I., Barca, D., van den Berg, S.A.A., Bertini, E., Brunner, D., Brunetti-Pierri, N., Cappa, M., et al. (2022). Long-term efficacy of T3 analogue Triac in children and adults with MCT8 deficiency: a real-life retrospective cohort study. J. Clin. Endocrinol. Metab. *107*, e1136–e1147. https://doi.org/10.1210/clinem/dgab750.

45. Nair, A.B., and Jacob, S. (2016). A simple practice guide for dose conversion between animals and human. J. Basic Clin. Pharm. *7*, 27–31. https://doi.org/10.4103/0976-0105.177703.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
6-propyl-2-thiouracil (PTU)	Tokyo Chemical Industry Co., Ltd.	Cat# P0533
3,3′,5-Triiodo-L-thyronine (LT3) sodium salt	Sigma-Aldrich	Cat# T6397
3,3′,5-Triiodothyroacetic acid (TRIAC)	Sigma-Aldrich	Cat# T7650
4% paraformaldehyde phosphate buffer solution	FUJIFILM Wako Pure Chemical Corporation	Cat# 163-20145
RNAprotect Tissue Reagent	QIAGEN	Cat# 76104
Radioimmunoprecipitation buffer	Nacalai Tesque	Cat# 08714-04
QIAzol Lysis Reagent	QIAGEN	Cat# 79306
Critical commercial assays		
Rodent TSH ELISA TEST KIT	Endocrine technologies, Inc.	Cat# ERKR7015
FT3, FT4, TSH AccuLite VAST CLIA kit	Monobind Inc.	Cat# 7075
Nucleospin RNA Plus kit	Macherey-Nagel	Cat# 740984
ReverTra Ace	TOYOBO Life Science	Cat# FSQ-201
THUNDERBIRD SYBR qPCR MIX	TOYOBO Life Science	Cat# QPS-201
Experimental models: Organisms/strains		
C57BL/6J mice	Japan SLC, Inc.	N/A
Oligonucleotides		
Primers for quantitative RT-PCR	This paper	See Table S9
Software and algorithms		
ImageJ	National Institutes of Health	https://imagej.nih.gov/ij/
JMP Pro version 16.1.0	SAS Institute Inc.	https://www.jmp.com/
Other		
Nexera X2 UHPLC	Shimadzu Corporation	Cat# Shimadzu-NX2-001
Raptor Biphenyl column, 2.1 × 50 mm, 1.8 μm	Restek	Cat# 5055-90159
Triple Quad™ 7500	AB SCIEX	https://www.sciex.com/products/mass-spectrometers/triple-quad-systems/
iMark microplate reader	Bio-Rad	https://www.bio-rad.com/en-jp/product/imark-microplate-absorbance-reader
Spark	Tecan	https://lifesciences.tecan.com/multimode-plate-reader
Triple Quad™ 5500+ system and QTRAP Ready	AB SCIEX	https://sciex.com/products/mass-spectrometers/triple-quad-systems/
InertSustain C18 column, 2.1 × 150 mm, 5 μm	GL Sciences	Cat# 5020-07315
StepOnePlus Real-Time PCR	Thermo Fisher Scientific	Cat# 4376598

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Ichiro Yamauchi (ichiroy@kuhp.kyoto-u.ac.jp).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This paper analyzes existing, publicly available data. This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Animals

Three-week-old C57BL/6J male mice were purchased from Japan SLC, Inc. (Hamamatsu, Japan). All experiments involving mice were approved by the Animal Research Committee, Graduate School of Medicine, Kyoto University (permit number: Med Kyo 21239). Animal care and experiments were conducted in accordance with our institutional guidelines. All animals were housed at 23°C in a 14:10-h light/dark cycle.

METHOD DETAILS

Chemical administration to mice and sample collection

PTU (Tokyo Chemical Industry Co., Ltd., Tokyo Japan), LT3 sodium salt (T6397; purity >95%; Sigma-Aldrich, St. Louis, MO), and TRIAC (T7650; purity >90%; Sigma-Aldrich) were purchased for administration to mice. Mice were administered the chemicals via drinking water from 3 to 7 weeks of age. Mice were housed in groups (4–6 mice per cage) and had *ad libitum* access to drinking water. Concentrations of PTU, LT3, and TRIAC used were 10 μg/mL or 100 μg/mL, 0.1 μg/mL or 1 μg/mL, and 0.1 μg/mL or 1 μg/mL, respectively. Drinking water containing chemicals was renewed every 3 days. Each vehicle group was administered with the same concentration of NaOH according to amount of stock solution of chemicals. Only when we evaluated recovery rate, bottles were wrapped with aluminum foil as protection from light exposure.

All mice were sacrificed by isoflurane exposure at the end of the light cycle. To obtain sera, blood samples were immediately collected from the inferior vena cava, left undisturbed for 45 min at room temperature, and subsequently cooled on ice. After centrifugation at 3,000 *g* for 15 min at 4°C, the supernatants were collected and stored at −80°C until use. Thyroid glands harvested for histological analyses were first fixed in 4% paraformaldehyde phosphate buffer solution (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan), embedded in paraffin, and stained using hematoxylin and eosin. Thyroid gland size was measured as thyroid area on photograph using ImageJ software (National Institutes of Health, Bethesda, MD). Lengths of tibia and lumbar vertebrae were measured on soft X-ray film. Pituitary glands and thyroid glands harvested for RNA extraction were preserved in RNAprotect Tissue Reagent (QIAGEN, Venlo, Netherlands) at −20°C until use. Livers, hearts, and cerebrums were collected in microtubes, immediately frozen in liquid nitrogen, and stored at −80°C until use. Phosphate buffered saline was used for perfusion of livers in the experiment regarding Figure S4.

Thyroid hormone measurement of serum

We measured serum total levels of T4, T3, and TRIAC using liquid chromatography-tandem mass spectrometry (LC-MS/MS).[18] Twenty microliters of each serum sample was mixed with 10 μL internal standard (IS) solution and 100 μL acetonitrile using a vortex mixer. The mixture was centrifuged at 15,000 rpm for 10 min at 4°C. T4-$^{13}C_6$ and T3-$^{13}C_6$ were used at 10 ng/mL as ISs for T4 and T3 measurements, respectively. For TRIAC measurements, T3-$^{13}C_6$ was used as the IS. Injection samples consisted of 60 μL of the supernatants and 240 μL of water. We injected 20 μL of each prepared sample into a Nexera X2 UHPLC system (Shimadzu Corporation, Kyoto, Japan) with a Raptor Biphenyl column (2.1 × 50 mm, 1.8 μm; Restek, Bellafonte, PA) maintained at 40°C. A gradient of mobile phase A (0.1% FA in water) and mobile phase B (methanol) was used. General conditions were as follows: 60% methanol (0–2.5 min), 60–80% methanol linear gradient (2.5–2.6 min), 80% linear gradient (2.6–4 min), 95% linear gradient (4–6 min), 60% linear gradient (6–8 min); flow rate of 0.4 mL/min (0–4 min), 0.5 mL/min (4–6 min), 0.4 mL/min (6–8 min). The following MS settings were adopted: curtain and collision gas pressure of 32 and 9 psi, respectively; ion spray voltage of 2500 V (positive mode) and −2000 V (negative mode); temperature of 450°C; and ion source gas 1 and 2 pressure of 40 and 70 psi; correspondingly. A Triple Quad 7500 system (AB SCIEX, Tokyo, Japan) was used to measure T4 and T3 with electrospray ionization (ESI) in positive mode and TRIAC with ESI in negative mode by multiple reaction monitoring (MRM). The limits of quantification of T4, T3, and TRIAC were all 0.01 ng/mL.

Serum TSH levels were evaluated using a Rodent TSH ELISA TEST KIT (Endocrine technologies, Inc., Newark, CA) and 20 μL of serum from each mouse. Absorbance was determined using an iMark microplate reader (Bio-Rad, Hercules, CA). We measured serum free T4 levels using an FT3, FT4, TSH AccuLite VAST CLIA kit (Monobind Inc., Lake Forest, CA) with Spark (Tecan, Zurich, Switzerland). This kit was also used for measuring LT3 concentration in drinking water to determine recovery rate.

Thyroid hormone measurement of organ

Organ-specific T4, T3, and TRIAC contents were measured using LC-MS/MS.[18] We mechanically homogenized 50 mg of either the liver or cerebrum of a mouse in 300 μL radioimmunoprecipitation buffer (Nacalai Tesque) and left the homogenate on ice for 30 min. Supernatants were centrifuged at 10,000 *g* for 10 min at 4°C and collected in microtubes. We added 300 μL methanol and 600 μL chloroform, mixed with vortex mixer, and centrifuged at 15,000 *g* for 2 min at 4°C. The upper water/methanol phase was collected in new microtubes. We injected 6 μL of each sample into a Triple Quad 5500+ system and QTRAP Ready (AB SCIEX). Chromatography was performed using InertSustain C18 column (2.1 × 150 mm, 5 μm; GL Sciences, Tokyo, Japan) maintained at 40°C. A gradient of mobile phase A (0.5 mM ammonium fluoride in water) and mobile phase B (methanol) was used. The general conditions were as follows: 40% methanol (0–1 min), 40–90% methanol linear gradient (1–10 min), 90% linear gradient (10–15 min); flow rate of 0.2 mL/min. The following MS settings were adopted: curtain and collision gas pressure of 40 and 8 psi, respectively; ion spray voltage of −4500 V; temperature of 500°C; and ion source gas 1 and 2 pressure of 80 and 70 psi; correspondingly. T4, T3, and TRIAC were all measured with ESI in negative mode by MRM. The limits of quantification of T4, T3, and TRIAC were all 0.01 ng/mL.

RNA extraction and quantitative RT-PCR

Total RNA was extracted using a Nucleospin RNA Plus kit (Macherey-Nagel, Düren, Germany) and reverse transcribed using ReverTra Ace (TOYOBO Life Science, Osaka, Japan). For cerebrums, we extracted total RNA using QIAzol Lysis Reagent (QIAGEN) and subsequently cleaned up with a Nucleospin RNA Plus kit. Quantitative RT-PCR was performed using THUNDERBIRD SYBR qPCR MIX (TOYOBO Life Science) with the StepOnePlus Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA). Results were normalized using means of *Ppia* and *Hprt* as the reference gene; relative mRNA expression of target genes was evaluated using the comparative threshold cycle method. Primer sequences used are listed in Table S9.

QUANTIFICATION AND STATISTICAL ANALYSIS

Results are expressed as means ± standard error of the mean and statistical analysis was performed using either Student's *t*-test or one-way analysis of variance followed by the Tukey-Kramer test. JMP Pro version 16.1.0 (SAS Institute Inc., Cary, NC) was used for all statistical analyses. Statistical significance was defined as a p value <0.05.