Inhibitory effects of methimazole and propylthiouracil on iodotyrosine deiodinase 1 in thyrocytes

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Abstract. Methimazole (MMI) and propylthiouracil (PTU) are commonly used for the treatment of Graves’ disease. They share similar inhibitory effects on thyroid hormone biosynthesis by interfering with thyroid peroxidase (TPO)-mediated oxidation and organization of iodide. However, their potential effects on other thyroid functional molecules have not been explored in depth. To identify novel effects of MMI and PTU, DNA microarray analysis, real-time PCR, Western blotting, immunofluorescence staining and confocal laser scanning microscopy were performed using FRTL-5 rat thyroid cells. DNA microarray analysis indicated that both MMI and PTU suppress iodotyrosine deiodinase 1 (Iyd, Dehal1) mRNA levels. Further studies revealed that Dehal1 mRNA levels was stimulated by TSH, insulin and serum, while it was suppressed by iodine and a follicular concentration of thyroglobulin. MMI and PTU significantly suppressed Dehal1 expression induced by TSH, insulin and serum. On the other hand, although MMI suppressed Dehal1 expression in the absence of TSH, PTU only weakly suppressed Dehal1 without TSH. These results suggest that PTU and MMI may use different mechanisms to regulate Dehal1 expression, and TSH may play essential and differential roles in mediating PTU and MMI signals in thyrocytes. The drugs also inhibited re-distribution of Dehal1 protein into newly formed lysosomes following thyroglobulin endocytosis. These findings imply complex and multifaceted regulation of Dehal1 in the thyroid and suggest that MMI and PTU modulate Dehal1 expression and distribution of the protein in thyrocytes to exert their effect.

Key words: Iodotyrosine deiodinase 1 (Dehal1), Methimazole (MMI), Propylthiouracil (PTU), Iodine, Thyroglobulin

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biosynthesis in balance, DEHAL1 has not been actively studied, and the results of previous studies have varied because of the poor stability and arduous purification of this protein. Thus, we explored the regulation of DEHAL1 expression in thyrocytes.

Material and Methods

Cell culture and treatment

FRTL-5 rat thyroid cells were grown in Coon's modified Ham's F12 medium supplemented with 5% bovine serum (Invitrogen, Carlsbad, CA, USA) and a mixture of six hormones (1 mU/mL TSH, 10 μg/mL insulin, 10 ng/mL somatostatin, 0.36 ng/mL hydrocortisone, 5 μg/mL transferrin and 2 ng/mL glycyl-L-histidyl-L-lysine acetate). In some experiments, cells were grown in this medium for 2 days followed by medium supplemented with 0.2% bovine serum without TSH and insulin for 5 days before use in experiments [10, 11]. MMI and PTU (both from Sigma Aldrich, St. Louis, MO, USA) were used at a final concentration of 5 mM as used in previous studies [12, 13].

DNA microarray analysis

FRTL-5 cells were treated with 5 mM MMI or PTU in the culture medium for 24 hours. Total RNA was purified using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA), and 1 μg total RNA was amplified using the Ambion Amino Allyl aRNA amplification kit (#1753, Ambion, Austin, TX, USA). The DNA microarray was provided by TORAY Industries, Inc. (Tokyo, Japan). Briefly, Cy5 labeling reactions were performed using Amersham Cy5 Mono-Reactive Dye (#PA25001, GE Healthcare, Buckinghamshire, UK). After purification, 1 μg each Cy5-labeled sample was hybridized to arrays in a hybridization chamber (#TX711, Takara Bio, Shiga, Japan) for 16 hours at 37°C. The arrays were scanned using the 3D-Gene Scanner 3000 (TORAY Industries).

Total RNA isolation and real-time PCR

Total RNA was purified using the RNeasy Plus Mini Kit (Qiagen), and cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Forester City, CA, USA) as described previously [10]. Quantitative real-time PCR was performed using the StepOne Plus Real-time PCR System (Applied Biosystems). A total of 20 ng cDNA mixed with 10 μL 2× Fast SYBR Green Master Mix (Applied Biosystems) was amplified by incubating for 20 minutes at 95°C, followed by 40 cycles of 3 seconds at 95°C and 30 seconds at 60°C. Each sample was measured in triplicate. The mRNA levels were normalized against those of Gapdh. The sequences of the PCR primers used were as follows: Dehail forward, 5'-TCCTATTCTCCACACCCCA-3'; Dehail reverse, 5'-GCTGTCTGCTGCTTTGATG-3'; Gapdh forward, 5'-ACAGCAAAGGCTGGTTGGAC-3'; and Gapdh reverse, 5'-TCTTGGGTTGCAGCGAATT-3'.

Western blot analysis

FRTL-5 cells were lysed in buffer containing 50 mM HEPES, 150 mM NaCl, 5 mM EDTA, 0.1% NP40 and 20% glycerol at 4°C for 1 hour, followed by addition of a protease inhibitor cocktail (Complete Mini, Roche Diagnostics, Basel, Switzerland). The mixture was centrifuged at 4°C for 20 minutes to recover total cellular proteins. Protein extracts were mixed with 4× lithium dodecyl sulfate sample buffer and 10× reducing agent (Invitrogen) and incubated at 70°C for 10 minutes prior to electrophoresis. The proteins were separated on a NuPAGE 4–12% Bis Tris gel by electrophoresis and transferred to iBlot2 PVDF Mini Stacks (Invitrogen). The membranes were washed with PBS supplemented with 0.1% Tween 20 (PBST) and blocked with PBST containing 5% nonfat milk for 1 hour. Membranes were then incubated with rabbit polyclonal anti-Gapdh (1:5,000, Cell Signaling Technology) or rabbit polyclonal anti-Ilyd (Dehail) primary antibody (1:500, Abcam, Cambridge, UK) at 4°C for 12 hours, followed by a donkey anti-rabbit IgG, HRP-linked secondary antibody (1:1,000, Cell Signaling Technology) at room temperature for 1 hour. HRP was visualized using Immunostar LD reagent (Wako Pure Chemical, Osaka, Japan), and chemiluminescence was detected using the C-Digit blot scanner (LI-COR, Lincoln, NE, USA).

Immunofluorescence staining

FRTL-5 cells were grown on poly-L-lysine-coated culture cover slips (Matsunami Glass, Osaka, Japan) in a 24-well plate and treated with 1 mg/mL bovine thyroglobulin (Tg) (Sigma Aldrich) and 5 mM MMI or PTU for 24 hours. To label lysosomes, LysoTracker Red DND-99 (Life Technologies) was added to the culture medium at a final concentration of 50 nM and incubated with the cells at 37°C for 1 hour. Cells were fixed with 10% buffered formalin (Wako Pure Chemical) for 15 minutes, permeabilized with 0.3% Triton X-100 (Wako Pure Chemical) in Dulbecco's Phosphate-Buffered Saline (DPBS) for 5 minutes, and blocked with 0.5% bovine serum albumin (Sigma Aldrich) in DPBS for 1 hour. Immunofluorescence staining of Dehail was performed by incubating the cover slips with rabbit polyclonal anti-Ilyd (1:500; Abcam) at 4°C overnight. Cover slips were incubated with Alexa Fluor 488-conjugated chicken anti-rabbit IgG antibody (1:1,000; Life Technologies) for 1 hour at room temperature. The nuclei were counterstained with Hoechst 333258 (1:1,000, Life Tech-
technologies), and coverslips were placed on glass slides with fluorescence mounting medium (Dako, Tokyo, Japan). Immunofluorescence was visualized, and the images were captured using the FV10i-LIV confocal laser scanning microscope (Olympus, Tokyo, Japan).

**Statistical analysis**

All experiments were repeated at least three times using different batches of cells, and the means ± SD were calculated. The significance of the differences between experimental values was determined by unpaired two-tailed t-tests, where \( p < 0.05 \) was considered to indicate significance.

**Results**

**MMI and PTU suppress Dehal1 expression**

To assess the overall effects of MMI and PTU on thyroid gene mRNA levels, we performed DNA microarray screening of candidate genes whose mRNA levels are modulated by MMI or PTU in thyrocytes. FRTL-5 cells maintained in growth medium were treated with 5 mM MMI or PTU for 24 hours. Total RNA was extracted from the cells and subjected to DNA microarray analysis. Overall, the results showed that 172 genes were upregulated and 353 downregulated more than twofold by MMI, while 192 genes were upregulated and 782 downregulated more than twofold by PTU (Fig. 1). The mRNA levels of well-established thyroid functional molecules, such as the sodium/iodide symporter (Nis; Slc5a5), thyroid peroxidase (Tpo), dual oxidase 2 (Duox2), and thyroid-specific transcription factors including thyroid transcription factor 1 (Ttf1; Nkx2-1), thyroid transcription factor 2 (Ttf2; Foxe1) and paired box gene 8 (Pax8), was not affected more than twofold by either MMI or PTU.

![DNA microarray analysis of FRTL-5 cells treated with anti-thyroid drugs.](image1)

**Fig. 1** DNA microarray analysis of FRTL-5 cells treated with anti-thyroid drugs. FRTL-5 cells were treated with 5 mM of MMI or PTU in the presence of TSH, insulin and 5% bovine serum for 24 hours. Total RNA was isolated and subjected to DNA microarray analysis. The expression levels of major thyroid functional genes were indicated as open circle. Other genes whose expression was changed more than two-fold (broken lines) were indicated as dots.

![MMI and PTU suppress Dehal1 mRNA levels.](image2)

**Fig. 2** MMI and PTU suppress Dehal1 mRNA levels. FRTL-5 cells were treated with 5 mM of MMI or PTU in the presence of TSH, insulin and 5% bovine serum for 48 hours. Total RNA was purified from the cells and subjected to real-time PCR analysis. Dehal1 mRNA levels normalized against that of Gapdh were expressed as fold change relative to control. Data are presented as mean ± SD relative to control levels (n = 3). **: \( p < 0.01 \) compared to control levels.

However, MMI and PTU suppressed mRNA levels of Dehal1, a key molecule responsible for iodine recycling, to 43% and 21% of the original levels, respectively (Fig. 1). To verify these suppressive effects of MMI and PTU on Dehal1, we performed real-time PCR analysis using mRNA derived from cells treated with MMI or PTU in growth medium up to 48 hours. In accordance with the DNA microarray analysis, real-time PCR demonstrated that Dehal1 expression was decreased to less than 50% of the original levels within 24 hours and remained decreased until 48 hours after treatment with MMI or PTU (Fig. 2).
Thyrocytes exhibit multifaceted regulation of Dehal1 expression

Other than one previous study reporting that Dehal1 mRNA levels was significantly induced by cyclic adenosine monophosphate (cAMP) [14], little is known regarding the regulation of Dehal1 in the thyroid. Therefore, before further exploring the effects of PTU and MMI, we examined the effects of essential hormonal regulators of thyroid functions (TSH, insulin and 5% serum) on Dehal1 mRNA levels in FRTL-5 cells by real-time PCR analysis. TSH, insulin and 5% serum each significantly induced Dehal1 mRNA levels in a time-dependent manner (Fig. 3A, 3B and 3C). A synergistic effect on Dehal1 induction was observed when the cells were stimulated with insulin and serum simultaneously, exceeding the contributions of each individual stimulus combined (Fig. 3E compared with 3B and 3C). However, when TSH was added simultaneously with serum (Fig. 3D) or with both insulin and serum (Fig. 3F), the insulin/serum-induced increase in Dehal1 mRNA was suppressed, indicating that TSH interferes with the signaling pathway(s) activated by insulin and/or serum to induce Dehal1 expression.

We then examined the effects of MMI and PTU combined with TSH or insulin. Induction of Dehal1 by TSH was almost completely abolished by MMI and PTU (Fig. 4). The stimulating effect of insulin on Dehal1 was completely abrogated by MMI, while PTU only weakly suppressed the effect of insulin on Dehal1 expression (Fig. 4), indicating that MMI and PTU have different effects on Dehal1 regulation.

Excess iodine modulates the accumulation and organification of iodine itself, putatively through negative regulation of NIS and TPO mRNA levels [15, 16]. To investigate whether excess iodine exerts its autoregulatory effect on iodine recycling by modulating Dehal1 expression, we treated FRTL-5 cells with sodium iodide (NaI). The results showed that NaI decreased Dehal1 expression in FRTL-5 cells (Fig. 5A). In addition to iodine, Tg protein at follicular concentrations has been recognized as a prominent negative-feedback autoregulator of follicular function [11, 17-19]. Thus, the expression levels of key thyroid functional genes, such as Tg, Slc5a5 and Tpo, are all subject to negative-feedback reg-
The rates of iodine uptake, oxidization and organification are not affected unilaterally by serum TSH but rather are tightly regulated by Tg accumulated in the follicular lumen [20, 21]. To investigate Tg action on iodine recycling, we examined *Dehal1* expression in cells treated with a follicular concentration of Tg and found that *Dehal1* expression was significantly suppressed by Tg (Fig. 5B), again suggesting that Tg is a significant autoregulator of the whole process of hormone biosynthesis in thyroid cells by suppressing iodine recycling.

**MMI and PTU inhibit redistribution of Dehal1 into lysosomes after endocytosis of Tg**

We performed Western blot analysis to examine the changes in Dehal1 protein levels in FRTL-5 cells treated with MMI or PTU for 24 hours. Total proteins were extracted from the cells and subjected to Western blot analysis using a rabbit anti-IYD (DEHAL1) antibody. In contrast to the suppression of the mRNA level, the total cellular protein level of 33 kDa Dehal1 was not significantly affected by MMI or PTU at 24 hours (Fig. 6). We thus investigated whether MMI and PTU prevent Dehal1 activation by inhibiting its subcellular translocation, rather than affecting its total protein level. To determine the subcellular localization of Dehal1 following Tg endocytosis, we treated FRTL-5 cells with a minimal amount of Tg (1 mg/mL) in the presence of MMI or PTU, labeled lysosomes with LysoTracker and performed immunofluorescence staining of Dehal1.
In the absence of Tg endocytosis, Dehal1 staining was diffuse throughout the cytoplasm, with the most pronounced staining in the vicinity of the nucleus, appearing granular (Fig. 7, Control). After the addition of Tg, newly formed lysosomes were observed and were colocalized mainly with Dehal1 (Fig. 7, Tg), indicating that lysosomal translocation of Dehal1 occurred in response to Tg endocytosis. By contrast, MMI and PTU treatment inhibited the entry of Dehal1 into lysosomes even though lysosome formation itself was not affected by these drugs (Fig. 7, Tg + MMI, Tg + PTU). Together, these results indicate that following Tg endocytosis, the Dehal1 protein enters newly formed lysosomes, putatively to initiate deiodination and facilitate rapid iodine recycling. MMI and PTU likely interfere with Dehal1-mediated iodine recycling by preventing Dehal1 from entering lysosomes to interact with its substrates (i.e., MIT and DIT), in addition to their suppressive effects on Dehal1 mRNA levels.

**Discussion**

Antithyroid drugs, also known as thionamides, have been the mainstay of treatment for Graves’ thyrotoxicosis since their original discovery in the 1940s as goitro-
gens in animals because of their pharmacologic inhibition of thyroid hormone production [22]. Currently, three such drugs are available: MMI, carbimazole and PTU. As carbimazole is rapidly metabolized into MMI and has all of the same properties as MMI [23], carbimazole and MMI are considered the same as far as mechanism of action is concerned. The chief actions of antithyroid drugs are inhibition of TPO-mediated iodine oxidation, iodine organification and iodotyrosine coupling [1], which contribute to inhibition of T3 and T4 biosynthesis. However, the effects of antithyroid drugs on thyroid functional molecules other than TPO remain to be explored. In the current study, we performed DNA microarray analysis to identify candidate genes modulated by antithyroid drugs as potential mediators of the drugs’ effects and identified Dehal1 as a target molecule of MMI and PTU in thyrocytes.

We showed that TSH, insulin and serum each induced Dehal1 mRNA levels. However, when added simultaneously with insulin and/or serum to treat cells, TSH suppressed the effects of both insulin and serum, indicating that opposing signaling pathways are possibly involved in the regulation of Dehal1 expression. It was suggested previously that TSH induces Dehal1 expression by increasing cAMP levels in thyrocytes [14]. However, insulin, which decreases cAMP levels [24], possibly regulates Dehal1 via a signaling pathway distinct from that activated by TSH/cAMP. Activation of second messengers and transcriptional regulation of Dehal1 gene need to be studied in the future. MMI inhibited both TSH- and insulin-induced elevation of Dehal1 mRNA, whereas PTU inhibited only TSH- but not insulin-induced Dehal1 expression. These results further suggest that different signaling pathways are involved in the regulation of Dehal1 by TSH versus insulin, and MMI and PTU may interfere with different steps of the signaling pathway that induces Dehal1. Overall, in the presence of TSH, insulin and serum, MMI and PTU significantly suppressed Dehal1 expression and its subcellular translocation, suggesting that these antithyroid drugs play novel roles in the regulation of Dehal1 to affect thyroid function.

In addition to the positive regulation of Dehal1 expression by TSH and insulin, our results suggest that Dehal1 is also subjected to negative-feedback regulation by iodine and Tg in thyrocytes. Excess NaI moderately suppressed Dehal1 in the presence of TSH and insulin, in support of a negative-feedback effect of iodine on iodine recycling. Follicular Tg serves as not only a hormone precursor but also a significant autoregulator, preventing the thyroid from overproducing thyroid hormones by modulating the rates of iodine uptake, oxidation and organification in a negative-feedback manner [18, 19, 21, 25]. We showed here that Tg at a follicular concentration almost abolished the induction of Dehal1 mRNA, suggesting that the negative-feedback effects of Tg extend to iodine recycling, again highlighting principle roles of follicular Tg in iodine homeostasis and hormone biosynthesis in the follicle.

In a previous study, in normal human thyroid tissues, immunostaining demonstrated localization of DEHAL1 in the cytoplasm of thyrocytes, with accumulation mostly at the apical membrane; this suggested that the deiodination reaction occurs at the apical pole of cells near the iodination site [14]. Probably because of the lack of polarity in FRTL-5 cell culture monolayers, the Dehal1 protein displayed greater intracytoplasmic rather than membrane localization and appeared to be most pronounced, often in a granular pattern, at the edge of the nucleus, as demonstrated by immunofluorescence. Following Tg endocytosis, the Dehal1 protein was found colocalized with newly formed lysosomes, where internalized Tg putatively undergoes degradation [26]. This re-distribution of Dehal1 following Tg endocytosis suggests that Dehal1-mediated iodine recycling occurs efficiently, together with Tg degradation in time- and spatial-dependent manners. By contrast, in MMI- or PTU-treated cells, Dehal1 protein remained diffusely located throughout the cytoplasm, instead of entering lysosomes, alluding to possible inactivation of Dehal1. An immunohistochemical study of Dehal1 in follicular and papillary thyroid cancer showed diffuse staining of DEHAL1 in the cytoplasm, without predominance in the apical membrane, compared with normal or benign thyroids [8]. Thus, the localization of DEHAL1 may be indicative of altered or lost DEHAL1 activity as a consequence of dedifferentiation or possibly antithyroid medications.

Iodinated Tg in the thyroid follicle re-enters thyrocytes by endocytosis and reaches the endosomes, where it undergoes lysosomal degradation to release T4 and T3 [27]. At the same time, large amounts of unused MIT and DIT are also released from Tg and then putatively interact with Dehal1 to undergo deiodination [14]. Proper maintenance of iodine in vivo requires not only its active transport into the thyroid but also its recovery from unused MIT and DIT formed in excess during hormone biosynthesis [3]. Free MIT and DIT that are estimated to be released 7-fold greater than T4 and T3, in a sense, represent a storehouse of iodine, which must be recovered by reductive dehalogenation catalyzed by DEHAL1 [3]; otherwise, the unused MIT and DIT would be excreted in the urine.

In summary, together our results imply that the regulation of Dehal1 in the thyroid is complex and multifaceted and is affected by stimulation with TSH, insulin and
serum. Meanwhile, Dehal1 expression was also subject to negative-feedback regulation by iodine and accumulat-
ed follicular Tg. MMI and PTU suppressed Dehal1 mRNA levels and likely affected the activity of the protein in thyocytes by preventing its lysosomal redistribution. Although the results need to be confirmed using human cells in the future, this study hopefully will shed light on hitherto unknown intrathyroidal effects of MMI and PTU on iodine recycling via Dehal1 regulation.

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Disclosure

None of the authors have any potential conflicts of interest associated with this research.

References

1. Davidson B, Soodak M, Neary JT, Stout HV, Kieffer JD, et al. (1978) The irreversible inactivation of thyroid peroxidase by methylmercaptoimidazole, thiouracil, and propylthiouracil in vitro and its relationship to in vivo findings. Endocrinology 103: 871–882.
2. Taurog A, Dorris ML (1989) A reexamination of the proposed inactivation of thyroid peroxidase in the rat thyroid by propylthiouracil. Endocrinology 124: 3038–3042.
3. Rokita SE, Adler JM, McTainney PM, Watson JA Jr (2010) Efficient use and recycling of the micronutrient iodide in mammals. Biochimie 92: 1227–1235.
4. Moreno JC, Klootwijk W, van Toor H, Pinto G, D’Alessandro M, et al. (2008) Mutations in the iodotyrosine deiodinase gene and hypothyroidism. N Engl J Med 358: 1811–1818.
5. Degroot LJ, Postel S, Litvak J, Stanbury JB (1958) Peptide-linked iodotyrosines and iodothyronines in the blood of a patient with congenital goiter. J Clin Endocrinol Metab 18: 158–166.
6. Burniat A, Pirson I, Vilain C, Kulik W, Afink G, et al. (2012) Iodotyrosine deiodinase defect identified via genome-wide approach. J Clin Endocrinol Metab 97: E1276–E1283.
7. Afink G, Kulik W, Overmars H, de Randamie J, Veenboer T, et al. (2008) Molecular characterization of iodotyrosine dehalogenase deficiency in patients with hypothyroidism. J Clin Endocrinol Metab 93: 4894–4901.
8. Krause K, Karger S, Gimm O, Sheu SY, Dralle H, et al. (2007) Characterisation of DEHAL1 expression in thyroid pathologies. Eur J Endocrinol 156: 295–301.
9. Thomas SR, McTainney PM, Adler JM, Laronde-Leblanc N, Rokita SE (2009) Crystal structure of iodotyrosine deiodinase, a novel flavoprotein responsible for iodide salvage in thyroid glands. J Biol Chem 284: 19659–19667.
10. Kawashima A, Tanigawa K, Akama T, Wu H, Sue M, et al. (2011) Fragments of genomic DNA released by injured cells activate innate immunity and suppress endocrine function in the thyroid. Endocrinology 152: 1702–1712.
11. Suzuki K, Lavaroni S, Mori A, Ohta M, Saito J, et al. (1998) Autoregulation of thyroid-specific gene transcription by thyroglobulin. Proc Natl Acad Sci U S A 95: 8251–8256.
12. Isozaki O, Tsushima T, Emoto N, Saji M, Tsuchiya Y, et al. (1991) Methimazole regulation of thyroglobulin biosynthesis and gene transcription in rat FRTL-5 thyroid cells. Endocrinology 128: 3113–3121.
13. Sue M, Akama T, Kawashima A, Nakamura H, Hara T, et al. (2012) Propylthiouracil increases sodium/iodide symporter gene expression and iodide uptake in rat thyroid cells in the absence of TSH. Thyroid 22: 844–852.
14. Gnidehou S, Caillou B, Talbot M, Ohayon R, Kaniecki J, et al. (2004) Iodotyrosine dehalogenase 1 (DEHAL1) is a transmembrane protein involved in the recycling of iodide close to the thyroglobulin iodination site. FASEB J 18: 1574–1576.
15. Eng PH, Cardona GR, Previti MC, Chin WW, Braverman LE (2001) Regulation of the sodium iodide symporter by iodide in FRTL-5 cells. Eur J Endocrinol 144: 139–144.
16. Uyttersprot N, Pelgrims N, Carrasco N, Gervy C, Maenhaut C, et al. (1997) Moderate doses of iodide in vivo inhibit cell proliferation and the expression of thyr
17. Suzuki K, Kawashima A, Yoshihara A, Akama T, Sue M, et al. (2011) Role of thyroglobulin on negative feedback autoregulation of thyroid follicular function and growth. J Endocrinol 209: 169–174.
18. Suzuki K, Kohn LD (2006) Differential regulation of apical and basal iodide transporters in the thyroid by thyroglobulin. J Clin Endocrinol Metab 193: 4984–4901.
19. Selliiti DF, Suzuki K (2014) Intrinsic regulation of thyroid function by thyroglobulin. Thyroid 24: 625–638.
20. Suzuki K, Mori A, Saito J, Moriyama E, Ullianech L, et al. (1999) Follicular thyroglobulin suppresses iodide uptake by suppressing expression of the sodium/iodide symporter gene. Endocrinology 140: 5422–5430.
21. Yoshihara A, Hara T, Kawashima A, Akama T, Tanigawa K, et al. (2012) Regulation of dual oxidase expression and H2O2 production by thyroglobulin. Thyroid 22: 1054–1062.
22. Mackenzie JB, Mackenzie CG, McCollum EV (1941) The effect of sulfanilylguanidine on the thyroid of the rat. Science 94: 518–519.
23. Jansson R, Dahlberg PA, Lindstrom B (1983) Compara-
24. Kitamura T, Kitamura Y, Kuroda S, Hino Y, Ando M, et al. (1999) Insulin-induced phosphorylation and activation of cyclic nucleotide phosphodiesterase 3B by the serine-threonine kinase Akt. *Mol Cell Biol* 19: 6286–6296.

25. Ishido Y, Luo Y, Yoshihara A, Hayashi M, Yoshida A, et al. (2015) Follicular thyroglobulin enhances gene expression necessary for thyroid hormone secretion. *Endocr J* 62: 1007–1015.

26. Oda K, Luo Y, Yoshihara A, Ishido Y, Sekihata K, et al. (2017) Follicular thyroglobulin induces cathepsin H expression and activity in thyrocytes. *Biochem Biophys Res Commun* 483: 541–546.

27. Marino M, McCluskey RT (2000) Role of thyroglobulin endocytic pathways in the control of thyroid hormone release. *Am J Physiol Cell Physiol* 279: C1295–C1306.