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Catalysis leads to post-translational inactivation of the Type 1 deiodinase and alters its conformation

Bo Zhu[*], **Ashutosh Shrivastava**[*], **Cristina Luongo**, **Ting Chen**, **John W. Harney**, **Alessandro Marsili**, **Thuy-Van Tran**, **Anulika Bhadouria**, **Radhika Mopala**, **Amanda I. Steen**, **P. Reed Larsen**, and **Ann Marie Zavacki**
Thyroid Section, Division of Endocrinology, Diabetes and Hypertension, Brigham and Women's Hospital, Boston, MA 02115, USA

Abstract

Previously it was shown that the type 1 deiodinase (D1) is subject to substrate dependent inactivation that is blocked by pretreatment with the inhibitor of D1 catalysis, propylthiouracil (PTU). Using HepG2 cells with endogenous D1 activity we found that while considerable D1-mediated catalysis of rT3 is observed in intact cells, there was a significant loss of D1 activity in sonicates assayed from the same cells in parallel. This rT3-mediated loss of D1 activity occurs despite no change in D1mRNA levels, and is blocked by PTU treatment, suggesting a requirement for catalysis. Endogenous D1 activity in sonicates was inactivated in a dose dependent manner in HepG2 cells, with a ~50% decrease after 10 nM rT3 treatment. Inactivation of D1 was rapid, occurring after only ½ hour of rT3 treatment. D1 expressed in HEK293 cells was inactivated by rT3 in a similar manner. ^{75}Se labeling of the D1 selenoprotein indicated that after 4 hours rT3-mediated inactivation of D1 occurs without a corresponding decrease in D1 protein levels, though rT3 treatment causes a loss of D1 protein after 8-24 hours. Bioluminescence Resonance Energy Transfer (BRET) studies indicate that rT3 exposure increases energy transfer between the D1 homodimer subunits, and this was lost when the active site of D1 was mutated to alanine, suggesting that a post-catalytic structural change in the D1 homodimer could cause enzyme inactivation. Thus, both D1 and type 2 deiodinase (D2) are subject to catalysis-induced loss of activity although their inactivation occurs *via* very different mechanisms.

Keywords

deiodinase; thyroid hormone action; thyroid hormone metabolism; selenoprotein

Introduction

The iodothyronine deiodinases are selenoenzymes that modulate T3 concentration by catalyzing both its production and degradation (Bianco, et al. 2002; Gereben, et al. 2008). T4 is activated *via* removal of an outer-ring iodine by the type 1 and 2 deiodinases (D1 and D2) to produce T3. Conversely, the type 3 deiodinase (D3), and under some conditions D1, can inactivate T3 and T4 by the elimination of an inner-ring iodine, generating T2 or reverse

Corresponding Author: Ann Marie Zavacki >Thyroid Section, Division of Endocrinology, Diabetes and Hypertension Brigham and Women's Hospital 77 Ave Louis Pasteur, HIM 645A Boston, MA 02155 azavacki@rics.bwh.harvard.edu Phone: 617-525-5153 Fax: 617-731-4718.
[*]These authors contributed equally to this work

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T3 (rT3). The deiodinases control circulating levels of thyroid hormone, with approximately 80% of the T3 produced daily in humans being derived extra-thyroidally from T4 *via* D1 and D2 (Bianco et al. 2002). Notably, in patients with a hyperactive thyroid gland, the contribution of thyroidal D1 to T4 to T3 conversion becomes predominant, with up to 2/3 of the daily T3 production coming from this source (Laurberg, et al. 2007). The deiodinases (primarily D2 and D3) also allow for intricate regulation of intracellular T3 concentrations in a tissue specific fashion independent of circulating concentrations of T4 or T3 (Bianco et al. 2002; Gereben et al. 2008).

Ubiquitination and subsequent proteasomal degradation of D2 are important components of the D2-mediated feedback regulation of TSH (reviewed in (Bianco et al. 2002; Gereben et al. 2008)). Ubiquitination of D2 is substrate dependent, increasing with catalysis of T4 (Steinsapir, et al. 1998; Steinsapir, et al. 2000). Thus, as more T4 is converted to T3, the ubiquitination and proteasomal degradation of D2 also increase, balancing T3 production. Additional layers of complexity are added to this scenario by the potential de-ubiquitination and reactivation of D2 by VDU1, and the finding that ubquitination drives apart the globular domain of the D2-homodimer, thus inactivating D2 yet also leaving the system primed for VDU1-mediated reactivation (Curcio-Morelli, et al. 2003b; Sagar, et al. 2007). Taken together, the substrate-mediated regulation of D2 activity provides a flexible mechanism to accurately regulate thyroid hormone production under a variety of physiological conditions.

D1 activity is also regulated by substrate exposure, though the physiological significance and mechanism of this have yet to be defined. D1 activity in liver microsomes was decreased when rats were injected with rT3, and D1 activity in Reuber FAO hepatoma cells was also reduced after rT3 treatment, although supraphysiological concentrations of rT3 were needed to achieve these effects (St Germain 1988a). Pretreatment with propylthiouracil (PTU) blocked the substrate-dependent loss of D1 activity in liver microsomes, suggesting that the loss of D1 activity was dependent upon catalysis (St Germain and Croteau 1989). The objective of this study was to further analyze the mechanism by which D1 is inactivated after substrate exposure. Using a human cell line, HepG2, with endogenous D1 expression, we find that while considerable D1-mediated catalysis of rT3 is observed in intact cells, there is a significant loss (68%) of D1 activity in cell sonicates. Similar results were found using a HEK293 system with transfected D1 and, notably, ^{75}Se labeling of the deiodinase selenoprotein indicated that initially rT3-mediated inactivation of D1 occurs despite stable D1 protein levels. Interestingly, Bioluminescence Resonance Energy Transfer (BRET) studies indicate that rT3 treatment increases energy transfer between the D1 homodimer subunits, suggesting that rT3 treatment either brings these monomers closer together, or stabilizes their interactions, and that this conformational change is associated with D1 inactivation.

Materials and methods

Materials

Chemicals and reagents were from Fisher Scientific (Pittsburgh, PA, USA) or Sigma (St. Louis, MO, USA) unless otherwise specified. ^{125}I rT3 (specific activity 4,400 Ci/mmol) was obtained from Perkin Elmer (Waltham, MA, USA), and ^{75}Se was a generous gift from Dr. Dolph Hatfield of the National Cancer Institute, Bethesda MD USA.

Cell Culture and Transfection

HepG2 or HEK293 cells were grown in DMEM + 10% FBS + 100 nM Se under 5% CO2. HEK293 cells were transfected with either a flag-tagged human D1 expression vector or a rat D1 expression vector where the serine at 128 was mutated to a proline and a vector

expressing β-galactosidase for normalization as described using Lipofectamine Plus (Invitrogen, Carlsbad, CA USA) (Callebaut, et al. 2003; Curcio-Morelli, et al. 2003a; Goemann, et al. 2010). Cells were treated with rT3 in DMEM + 0.1% BSA for the indicated time period. Transfected HEK293 cells were maintained in 6-well dishes and treated with 1.5 ml media containing the indicated agents.

Deiodinase assays

Assessment of D1 activity in whole cells and in sonicates was performed as described previously (Goemann et al. 2010). For sonicates, 2-50 μg of protein was incubated 1 hour at 37°C in the presence of 1 μM unlabeled rT3 and tracer amounts of ^{125}I rT3 and 10 mM DTT, followed by TCA precipitation and counting of the $^{125}I^-$ released in the supernatant. Under these conditions ~50 % of the D1 can be recovered from the D1-PTU complex from cells pre-treated with PTU. Activity from transfected HEK293 cells was normalized by β-galatosidase activity to control for transfection efficiency. Non-specific iodide release was determined in HepG2 cells by the addition of 10 mM PTU, and in HEK 293 cells by the assay of lysates from untransfected cells. Whole-cell D1 activity was measured in HepG2 cells by incubation of cells with 1 μM unlabeled rT3 and tracer amounts of ^{125}I rT3 in DMEM + 0.1% BSA for 20 hours followed by TCA precipitation of media and counting of the $^{125}I^-$ remaining in the supernatant. Non-specific whole cell deiodination was determined by the addition of 100 μM PTU.

Bioluminescence Resonance Energy Transfer (BRET) measurement

Energy transfer between carboxyl-terminal tagged renilla Luciferase-D1 and YPF-D1 fusion proteins was assessed as described previously (Sagar et al. 2007). Luciferase-D1 and YFP-D1 proteins where Sec126 was changed to Ala were created by amplifying BG132 (a D1 expression vector with Ala126 (Curcio-Morelli et al. 2003a)) with Pfu turbo polymerase (Stratagene) using the following oligos: 5′-ACGGATTCATTATGGGGCTGTCCCAGCTATG-3′ and 5′-TAGTGGATCCCGGAACTGAGGCATGTGTCC-3′. This fragment was cut with EcoRI and BamHI and subcloned into the carboxy-terminal tagged Luciferase-D1 and YFP-D1 expression vectors. All constructs were sequenced to confirm mutations and fusion reading frame.

^{75}Se labeling of D1

35 mm dishes of HEK 293 cells were transfected with 200 ng of a human D1 expression vector (Curcio-Morelli et al. 2003a), and ^{75}Se labeled as described previously in 1 ml of DMEM + 10% FBS + 50 nM Na_2SeO_3 and + 2 μCi of $Na\ ^{75}{}_2SeO_3$ (Curcio, et al. 2001). After 20 hours of labeling, cells were treated with 10 μM rT3 in DMEM + 0.1% BSA for the indicated time period, lysed, and 20 ug of each sample used for SDS-PAGE on 10% gel followed by autoradiography.

mRNA preparation and quantitative real-time PCR

mRNA was harvested from HepG2 cells using Trizol (Invitrogen, Carlsbad, CA, USA), and quantitative real time PCR was performed as describe previously (Zavacki, et al. 2005). Primer sequences for human D1 are: 5′-ACATCAGAAATCACCAGAACCTTCA-3′and 5′-CCAGAACAGCACGAACTTCCTC-3′, for human β-actin: 5′-GGCACCACACCTTCTACAATGAG-3′ and 5′- CCAGAGGCGTACAGGGATAGC-3′.

Results

Whole cell D1-mediated deiodination of 1 μM rT3 in the presence of tracer amounts of ^{125}I rT3 was determined in HEPG2 cells overnight, with this activity being completely blocked by the addition of 100 μM PTU (Fig 1A). However, when D1 activity was measured in cell sonicates from the same plates in the presence of 10 mM DTT, the activity in the PTU-treated sonicates was almost 3-times that of sonicates from plates without PTU treatment (Fig. 1B). PTU-treatment was also protective of D1 activity when HEK293 cells transfected with D1 were rT3 treated, leading to a 2-fold greater activity in sonicates (Fig. 1C). A conserved serine at position 128 found two amino acids downstream from the active site selenocysteine of D1 is essential for PTU sensitivity (Sanders, et al. 1997; Callebaut et al. 2003; Kuiper, et al. 2006). When this serine was changed to proline, as is found in the PTU insensitive Tilapia and Xenopus D1, and the D2 and D3 enzymes, the activity of the Ser128Pro D1 was no longer inhibited by PTU (Sanders et al. 1997; Callebaut et al. 2003; Kuiper et al. 2006). Consistent with this loss of sensitivity, PTU no longer prevented the rT3-induced decrease in activity of the Ser128Pro D1 enzyme (Fig. 1D).

D1 activity in HepG2 sonicates was reduced by rT3 treatment in a dose dependent manner, decreasing by ~50% after overnight treatment with 10 nM rT3, and by ~75% in the presence of 1 μM rT3 (Fig. 2A). This decrease in activity could not be explained by carry over of unlabeled substrate since only 1.7% of the radiolabled ^{125}I rT3 tracer was present in sonicates. Since only a small fraction of the total cell sonicate is used for deiodinase assays, even at the highest rT3 concentrations used this carry over would only account for ~2.8% of the final rT3 concentration used in the *in vitro* deiodinase reaction. The substrate-dependent inactivation of endogenous D1 in HepG2 cells was rapid, occurring within 30 minutes of rT3 exposure (Fig. 2B). Importantly, D1 mRNA levels in HepG2 cells were unchanged after 20 hours of rT3 treatment, despite a 66% decrease in activity in plates treated in parallel (Fig. 3A,B).

A similar substrate-mediated inactivation of D1 occurred when D1 was transiently expressed. D1 activity in sonicates from HEK293 cells transfected with human D1 was also decreased by rT3 treatment, although the amount of rT3 needed was ~10-times greater than that required to inactivate endogenous D1 (Compare Fig. 2A, 4A). rT3 treatment also significantly decreased activity of transiently expressed D1 in a time dependent fashion, with activity being decreased by ~50% after 4 hours of treatment with a continued decrease thereafter (Fig. 4B) ($p < 0.001$ by two-way ANOVA). Notably, when levels of the D1 selenoprotein were monitored by ^{75}Se labeling there was not a corresponding decrease in D1 protein after 4 hours (Fig 4B,C). However, D1 protein levels markedly decrease by 8 hours of treatment, and after 24 hours of rT3 exposure ^{75}Se labeled D1 is no longer apparent (Fig 4C). Similar results were obtained when the effect of rT3 on transfected flag-tagged D1 was studied by Western blotting using α-flag, with less D1 protein being apparent 8 and 24 hours after rT3 treatment (data not shown). Taken together, this suggests that rT3 treatment is accelerating the degradation of the D1 protein and not merely causing selective loss of its selenium. Activity of another deiodinase family member, the type 2 deiodinase (D2), is also subject to substrate-induced inactivation *via* ubiquitination and proteasomal degradation (Steinsapir et al. 1998; Steinsapir et al. 2000). However, treatment of D1-transfected HEK293 cells with the proteasomal inhibitor MG132 did not prevent the loss of D1 activity after 24 hours of rT3 treatment, confirming previous data that the mechanism for the loss of D1 activity is distinct from that of D2 (data not shown) (Gereben, et al. 2000). In addition, ubiquitination of D1 is further ruled out by the absence of an increase in molecular weight of the ^{75}Se labeled D1 (data not shown).

To determine if there could be structural modifications of the D1 protein during catalysis that could affect its homodimerization, we determined the Bioluminescence Resonance Energy Transfer (BRET) between a D1 protein tagged at the carboxy terminus with renilla Luciferase and a D1 protein tagged in the carboxy terminus with yellow fluorescent protein (YFP). We confirmed that rT3-treatment leads to the inactivation of both D1 fusion proteins in cell sonicates (Fig 5A,B). Notably, rT3 treatment increases the BRET signal between the D1 dimers, suggesting that exposure to substrate results in a molecular re-arrangement that either brings the luciferase and YFP moieties into closer proximity, or stabilizes the interactions between the D1 dimers (Fig 5C). Interestingly, the increase in BRET signal persists for at least an hour after cells were washed to remove the rT3 (data not shown).

To assess if catalysis of rT3 was necessary to mediate an increase in BRET signal with rT3 treatment, we evaluated the effects of changing the active center of the D1-YFP and D1-renilla Luciferase to alanine. While BRET still exists between the subunits of the D1 dimer with alanine in the active center, there was no longer an increase in BRET after rT3 treatment (Fig 5D). Further, no increase in BRET was observed when even one of the BRET partners had the active center mutated to an alanine, suggesting both partners of the D1 dimer must be catalytically active for the increase in BRET signal observed with rT3 treatment to occur (data not shown).

Discussion

Our results indicate that D1 activity in cells treated with rT3 is deceased in a rapid and dose-dependent manner (Fig. 2, Fig. 4). Furthermore, assessment of whole-cell mediated deiodination in HepG2 cells indicated that prior to substrate dependent inactivation of D1, substantial D1-mediated deiodination of rT3 occurs, consistent with catalysis preceding inactivation (Fig. 1A,B). Our data agrees with previous work from St. Germain's group where D1 activity was decreased in rat microsomes and in Ruber FAO hepatoma cells after treatment with either IOP or rT3 (St Germain 1988a). In addition, this effect was prevented by pre-treatment with the D1 inhibitor PTU, also supporting a requirement for catalysis (St Germain and Croteau 1989). PTU blocks D1 activity by competing with an endogenous thiol cofactor for a putative selenenyl-iodine intermediate (E-Se-I) blocking a full cycle of catalysis, and thus protecting D1 from rT3-mediated inactivation (Leonard and Visser 1986; St Germain and Croteau 1989). Further, after PTU treatment *in vivo*, D1 activity can be recovered by DTT treatment *in vitro*, indicating that the D1-PTU interaction can be disrupted by DTT and an active enzyme regenerated (St Germain and Croteau 1989). Thus, in cells with rT3-treatment alone, the D1 enzyme is inactivated, while in the rT3- and PTU-treated group (where full catalysis of rT3 is prevented by PTU) D1 activity can be recovered since our deiodinase assays are performed in the presence of 10 mM DTT (Fig. 1 B,C). Notably, when the PTU-insensitive Ser128Pro D1 mutant is treated with rT3 ± PTU, PTU treatment no longer blocks the rT3-mediated decrease in D1 activity, further supporting that PTU's inhibitory effects on D1 catalysis are necessary for its protective effects (Fig. 1D) (Callebaut et al. 2003).

No change in D1 mRNA levels was observed in rT3-treated HepG2 cells, indicating that the observed loss of D1 activity is not due to decreased *Dio1* expression or accelerated mRNA degradation (Fig. 3). Consistent with the post-transcriptional nature of these effects, rT3 treatment also decreased D1 activity in a transfected cell system where D1 was expressed from a heterologous promoter (Fig. 4A,B). Approximately 10-times more rT3 was required to decrease D1 activity in a transfected system, and this difference persisted even when transfection conditions were adjusted such that enzyme activity levels were decreased by 4-fold (data not shown) (Fig. 4A). However, we cannot rule out that this difference might be due to the increased levels of D1 enzyme in a transient versus endogenous expression

system. Alternatively, rT3 can also be specifically transported inside the cell *via* thyroid hormone transporters and thus potential differences in transporter expression between the HepG2 and the HEK293 cells used for these experiments might also contribute to the observed differences (van der Deure, et al. 2008a; van der Deure, et al. 2008b; Kinne, et al. 2010).

Our results show that the mechanism of D1 inactivation by rT3 is post-translational, since even at 4 hours after rT3 treatment, when D1 activity is decreased by 50%, no corresponding decrease in ^{75}Se D1 protein is evident (Fig. 4B,C). The D1 enzyme has a long half-life that is estimated to be greater than 6 hours, and this was significantly decreased by rT3 treatment in Reuber FAO hepatoma cells (St Germain 1988a; Baqui, et al. 2003). Our data also indicate that rT3 accelerates the degradation of D1 protein, since after 24 hours ^{75}Se labeled D1 can still be detected in the vehicle treated group, while none is apparent in group treated with rT3 in parallel (Fig. 4C). A rT3-enhanced loss of D1 protein over time is consistent with previous results indicating that the substrate-inducible loss of D1 is reversible early time points after treatment, but becomes only partially reversible after longer periods (St Germain and Croteau 1989).

The ubiquitination and subsequent degradation of D2 is substrate dependent, increasing with catalysis of T4 to provide a flexible system where T3 production is counter-balanced by a loss of D2 protein (Steinsapir et al. 1998; Steinsapir et al. 2000). Unlike D2, D1 does not appear to be regulated by ubiquitination. Using a transient expression system with a D1 enzyme where the active site selenocysteine was converted to a cysteine to facilitate overexpression, we did not observe any higher molecular weight forms of D1 by Western blotting indicative of ubiquitination (Gereben et al. 2000). ^{75}Se labeled wild-type D1 enzyme also did not display the characteristic ladder of higher molecular weight forms associated with ubiquitination, nor does MG132 treatment with the proteasomal inhibitor block the substrate-dependent decrease in D1 activity, further supporting that D1 is not regulated by the ubiquitin-proteasomal system.

While the T4-mediated decrease in D2 activity has been shown to occur under physiologically relevant conditions, the lowest concentration of rT3 where substrate dependent inactivation of D1 can be observed in HepG2 cells is 10 nM rT3, or 1 μM rT3 in a transfected cell system (Fig. 2) (Silva and Larsen 1982; Leonard, et al. 1984; St Germain 1988b; Steinsapir et al. 1998). Total rT3 concentrations range from 0.14 to 0.34 nmol/L in normal individuals, indicating that the concentrations needed to inactivate D1 are several orders of magnitude above circulating amounts, and thus the physiological implications of D1 inactivation are currently unclear (Peeters, et al. 2003). However, notably, another D1 substrate, T4, has circulating concentrations that are several orders of magnitude greater than rT3. Remarkably, a similar paradigm can be observed for D3, with exposure to high amounts of substrate (10 μM T3) resulting in D3 inactivation in a transient expression system (unpublished data B Zhu, PR Larsen, and AM Zavacki).

The deiodinase enzymes are homodimers, and BRET studies have shown that T4-dependent ubquitination separates the globular domains of the D2-homodimer, resulting in a loss of D2 activity (Leonard, et al. 2001; Curcio-Morelli et al. 2003a; Sagar et al. 2007; Sagar, et al. 2008). In contrast, we find that BRET signal between D1-YFP and D1-luciferase homodimers is increased after rT3 exposure, suggesting either a conformational change that positions the YFP and luciferase moieties into closer proximity, or a stabilization of homodimerization, resulting in increased signal (Fig. 5C). This correlates with the loss of D1 activity, suggesting that a conformational change in D1 homodimerization results in the loss of D1 activity after rT3 treatment. When the active center of D1 was changed to

alanine, rT3 treatment no longer caused an increase in BRET, indicating that catalysis is required to induce this conformational change (Fig. 5D).

Notably, the substrate-dependent inactivation of D1 occurs despite clearly present D1 protein (Fig. 4C). However, no observable change in D1 mobility was observed under reducing SDS-PAGE conditions, suggesting no covalent modifications are associated with this loss (Fig. 4C). The loss of D1 activity could be observed using both a wild type D1 enzyme, and an enzyme where the active site selenocysteine had been changed to cysteine (Fig. 2, 4, 5), indicating that the loss of D1 activity is not dependent upon the presence of a selenocysteine residue in its active center. The Sec126Cys D1 has been shown to have a lower affinity for rT3 that wild type D1, however surprisingly, when increasing doses of rT3 were tested for their ability to confer inactivation, the Sec126Cys D1 enzyme was somewhat more sensitive to rT3-mediated loss of D1 activity (data not shown) indicating that substrate-dependent inactivation cannot be solely predicted by the affinity for substrate in the presence of DTT *in vitro* (Berry, et al. 1992).

Our results provide further support for a requirement for catalysis in the substrate dependent inactivation of D1. Previously it has been suggested that exposure to saturating substrate concentrations oxidizes the D1 enzyme, although it is puzzling as to why the D1 enzyme cannot be adequately regenerated by endogenous co-factors within the cell (St Germain 1988b; St Germain and Croteau 1989; Croteau, et al. 1998; Wajner, et al. 2011). Our results support that whatever the change in the D1 enzyme, this inactivation occurs despite persistent D1 protein levels and is accompanied by a conformational change, and that this modification further results in increased degradation of the D1 enzyme over time. While it has been well established that D2 activity is decreased by exposure to substrate, these data and our preliminary studies with D3 indicate that this appears to be a general mechanism applicable to all three deiodinases.

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References

Baqui M, Botero D, Gereben B, Curcio C, Harney JW, Salvatore D, Sorimachi K, Larsen PR, Bianco AC. Human type 3 iodothyronine selenodeiodinase is located in the plasma membrane and undergoes rapid internalization to endosomes. J Biol Chem. 2003; 278:1206–1211. [PubMed: 12419801]

Berry MJ, Maia AL, Kieffer JD, Harney JW, Larsen PR. Substitution of cysteine for selenocysteine in type I iodothyronine deiodinase reduces the catalytic efficiency of the protein but enhances its translation. Endocrinology. 1992; 131:1848–1852. [PubMed: 1396330]

Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocr Rev. 2002; 23:38–89. [PubMed: 11844744]

Callebaut I, Curcio-Morelli C, Mornon JP, Gereben B, Buettner C, Huang S, Castro B, Fonseca TL, Harney JW, Larsen PR, et al. The iodothyronine selenodeiodinases are thioredoxin-fold family proteins containing a glycoside hydrolase clan GH-A-like structure. J Biol Chem. 2003; 278:36887–36896. [PubMed: 12847093]

Croteau W, Bodwell JE, Richardson JM, St Germain DL. Conserved cysteines in the type 1 deiodinase selenoprotein are not essential for catalytic activity. J Biol Chem. 1998; 273:25230–25236. [PubMed: 9737986]

Curcio C, Baqui MM, Salvatore D, Rihn BH, Mohr S, Harney JW, Larsen PR, Bianco AC. The human type 2 iodothyronine deiodinase is a selenoprotein highly expressed in a mesothelioma cell line. J Biol Chem. 2001; 276:30183–30187. [PubMed: 11425850]

Curcio-Morelli C, Gereben B, Zavacki AM, Kim BW, Huang S, Harney JW, Larsen PR, Bianco AC. In vivo dimerization of types 1, 2, and 3 iodothyronine selenodeiodinases. Endocrinology. 2003a; 144:937–946. [PubMed: 12586771]

Curcio-Morelli C, Zavacki AM, Christofollete M, Gereben B, de Freitas BC, Harney JW, Li Z, Wu G, Bianco AC. Deubiquitination of type 2 iodothyronine deiodinase by von Hippel-Lindau protein-interacting deubiquitinating enzymes regulates thyroid hormone activation. J Clin Invest. 2003b; 112:189–196. [PubMed: 12865408]

Gereben B, Goncalves C, Harney JW, Larsen PR, Bianco AC. Selective proteolysis of human type 2 deiodinase: a novel ubiquitin-proteasomal mediated mechanism for regulation of hormone activation. Mol Endocrinol. 2000; 14:1697–1708. [PubMed: 11075806]

Gereben B, Zavacki AM, Ribich S, Kim BW, Huang SA, Simonides WS, Zeold A, Bianco AC. Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. Endocr Rev. 2008; 29:898–938. [PubMed: 18815314]

Goemann IM, Gereben B, Harney JW, Zhu B, Maia AL, Larsen PR. Substitution of serine for proline in the active center of type 2 iodothyronine deiodinase substantially alters its in vitro biochemical properties with dithiothreitol but not its function in intact cells. Endocrinology. 2010; 151:821–829. [PubMed: 19966180]

Kinne A, Kleinau G, Hoefig CS, Gruters A, Kohrle J, Krause G, Schweizer U. Essential molecular determinants for thyroid hormone transport and first structural implications for monocarboxylate transporter 8. J Biol Chem. 2010; 285:28054–28063. [PubMed: 20628049]

Kuiper GG, Klootwijk W, Morvan Dubois G, Destree O, Darras VM, Van der Geyten S, Demeneix B, Visser TJ. Characterization of recombinant Xenopus laevis type I iodothyronine deiodinase: substitution of a proline residue in the catalytic center by serine (Pro132Ser) restores sensitivity to 6-propyl-2-thiouracil. Endocrinology. 2006; 147:3519–3529. [PubMed: 16601143]

Laurberg P, Vestergaard H, Nielsen S, Christensen SE, Seefeldt T, Helleberg K, Pedersen KM. Sources of circulating 3,5,3′-triiodothyronine in hyperthyroidism estimated after blocking of type 1 and type 2 iodothyronine deiodinases. J Clin Endocrinol Metab. 2007; 92:2149–2156. [PubMed: 17389703]

Leonard JL, Silva JE, Kaplan MM, Mellen SA, Visser TJ, Larsen PR. Acute posttranscriptional regulation of cerebrocortical and pituitary iodothyronine 5′-deiodinases by thyroid hormone. Endocrinology. 1984; 114:998–1004. [PubMed: 6697974]

Leonard, JL.; Visser, TJ. Thyroid Hormone Metabolism. Hennemann, G., editor. Marcel Dekker; New York: 1986. p. 189-229.

Leonard JL, Visser TJ, Leonard DM. Characterization of the subunit structure of the catalytically active type I iodothyronine deiodinase. J Biol Chem. 2001; 276:2600–2607. [PubMed: 11044448]

Peeters RP, Wouters PJ, Kaptein E, van Toor H, Visser TJ, Van den Berghe G. Reduced activation and increased inactivation of thyroid hormone in tissues of critically ill patients. J Clin Endocrinol Metab. 2003; 88:3202–3211. [PubMed: 12843166]

Sagar GD, Gereben B, Callebaut I, Mornon JP, Zeold A, Curcio-Morelli C, Harney JW, Luongo C, Mulcahey MA, Larsen PR, et al. The thyroid hormone-inactivating deiodinase functions as a homodimer. Mol Endocrinol. 2008; 22:1382–1393. [PubMed: 18356288]

Sagar GD, Gereben B, Callebaut I, Mornon JP, Zeold A, da Silva WS, Luongo C, Dentice M, Tente SM, Freitas BC, et al. Ubiquitination-induced conformational change within the deiodinase dimer is a switch regulating enzyme activity. Mol Cell Biol. 2007; 27:4774–4783. [PubMed: 17452445]

Sanders JP, Van der Geyten S, Kaptein E, Darras VM, Kuhn ER, Leonard JL, Visser TJ. Characterization of a propylthiouracil-insensitive type I iodothyronine deiodinase. Endocrinology. 1997; 138:5153–5160. [PubMed: 9389495]

Silva JE, Larsen PR. Comparison of iodothyronine 5′-deiodinase and other thyroid-hormone-dependent enzyme activities in the cerebral cortex of hypothyroid neonatal rat. Evidence for adaptation to hypothyroidism. J Clin Invest. 1982; 70:1110–1123. [PubMed: 6215429]

St Germain DL. Dual mechanisms of regulation of type I iodothyronine 5′-deiodinase in the rat kidney, liver, and thyroid gland. Implications for the treatment of hyperthyroidism with radiographic contrast agents. J Clin Invest. 1988a; 81:1476–1484.

St Germain DL. The effects and interactions of substrates, inhibitors, and the cellular thiol-disulfide balance on the regulation of type II iodothyronine 5′-deiodinase. Endocrinology. 1988b; 122:1860–1868. [PubMed: 3359966]

St Germain DL, Croteau W. Ligand-induced inactivation of type I iodothyronine 5′-deiodinase: protection by propylthiouracil in vivo and reversibility in vitro. Endocrinology. 1989; 125:2735–2744. [PubMed: 2792006]

Steinsapir J, Bianco AC, Buettner C, Harney J, Larsen PR. Substrate-induced down-regulation of human type 2 deiodinase (hD2) is mediated through proteasomal degradation and requires interaction with the enzyme's active center. Endocrinology. 2000; 141:1127–1135. [PubMed: 10698189]

Steinsapir J, Harney J, Larsen PR. Type 2 iodothyronine deiodinase in rat pituitary tumor cells is inactivated in proteasomes. J Clin Invest. 1998; 102:1895–1899. [PubMed: 9835613]

van der Deure WM, Friesema EC, de Jong FJ, de Rijke YB, de Jong FH, Uitterlinden AG, Breteler MM, Peeters RP, Visser TJ. Organic anion transporter 1B1: an important factor in hepatic thyroid hormone and estrogen transport and metabolism. Endocrinology. 2008a; 149:4695–4701. [PubMed: 18499754]

van der Deure WM, Hansen PS, Peeters RP, Kyvik KO, Friesema EC, Hegedus L, Visser TJ. Thyroid hormone transport and metabolism by organic anion transporter 1C1 and consequences of genetic variation. Endocrinology. 2008b; 149:5307–5314. [PubMed: 18566113]

Wajner SM, Goemann IM, Bueno AL, Larsen PR, Maia AL. IL-6 promotes nonthyroidal illness syndrome by blocking thyroxine activation while promoting thyroid hormone inactivation in human cells. J Clin Invest. 2011; 121:1834–1845. [PubMed: 21540553]

Zavacki AM, Ying H, Christoffolete MA, Aerts G, So E, Harney JW, Cheng SY, Larsen PR, Bianco AC. Type 1 iodothyronine deiodinase is a sensitive marker of peripheral thyroid status in the mouse. Endocrinology. 2005; 146:1568–1575. [PubMed: 15591136]

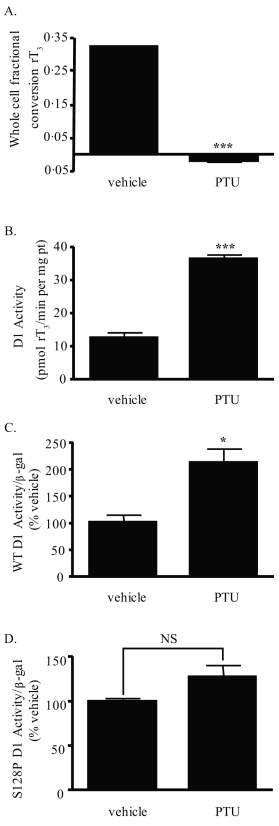

Fig. 1.

PTU treatment inhibits the rT3-mediated loss of D1 activity in HepG2 cells, and in D1-transfected HEK293 cells. (A) HepG2 cells were incubated for 20 hours with 1μM rT3 and tracer amounts of ^{125}I rT3± 100 μM PTU and whole cell D1-mediated deiodination was determined. (B) Cells from (A) were harvested and D1 activity measured in sonicates in the presence of 10 mM DTT. (C, D) HEK293 cells were transfected with vectors expressing either a wild type D1 (C) or a Ser128Pro D1 (D) and β-galactosidase. Cells were treated with 3 μM rT3 ± 100 μM PTU as indicated for 20 hours, and D1 activity was measured in sonicates and normalized by β-galactosidase activity. Data are normalized to the vehicle treated control group being 100% for C and D. The mean ± SEM of 3 wells/group are shown with similar results being obtained in 2-3 experiments. ***=p<0.001, *=p<0.05 by student's unpaired t-test, *ns*=non-significant.

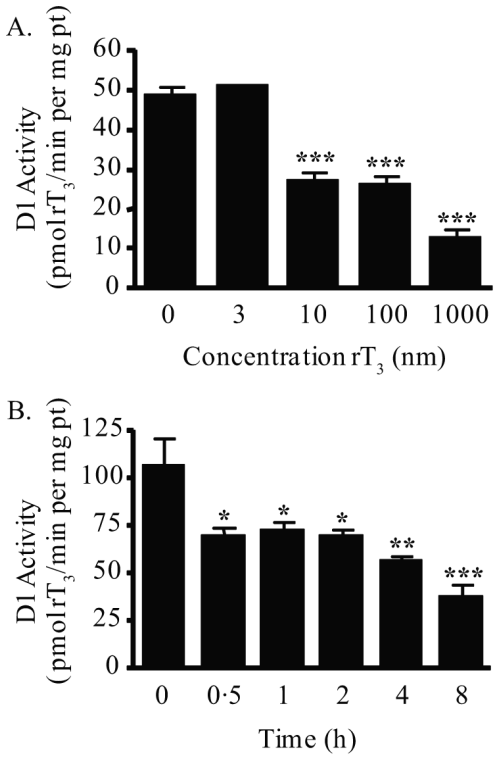

Fig. 2.

Dose response and time course of inhibition of D1 activity in HepG2 cells by rT3 exposure. (A) HepG2 cells were treated with the indicated concentrations of rT3 for 20 hours and D1 activity in sonicates was measured in the presence of 10 mM DTT. (B) HepG2 cells were treated with 1 μM rT3 for the indicated times and then D1 activity measured as in (A). The mean ± SEM of 3 wells/group are shown, with similar results being obtained in 3 experiments. ***=p<0.001, **=p<0.001, *=p<0.05 by one-way ANOVA when compared to either the vehicle control (A) or time 0 control (B) group.

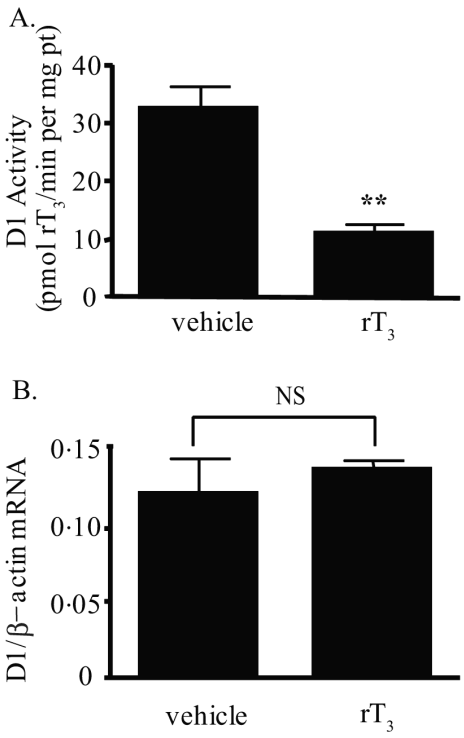

Fig. 3.

D1 mRNA levels in HepG2 cells after rT3 treatment. HepG2 cells were treated with 1μM rT3 for 20 hours before mRNA harvest. (A) D1 activity was determined or (B) D1 mRNA levels were measured by quantitative real-time PCR and are shown normalized by the expression of the housekeeping gene β-actin. Plates for A and B were treated at the same time within the same experiment. The mean ± SEM of 3 wells/group are shown, with similar results being obtained in 3 experiments. **=p<0.01 by student's unpaired t-test, *ns*=non-significant.

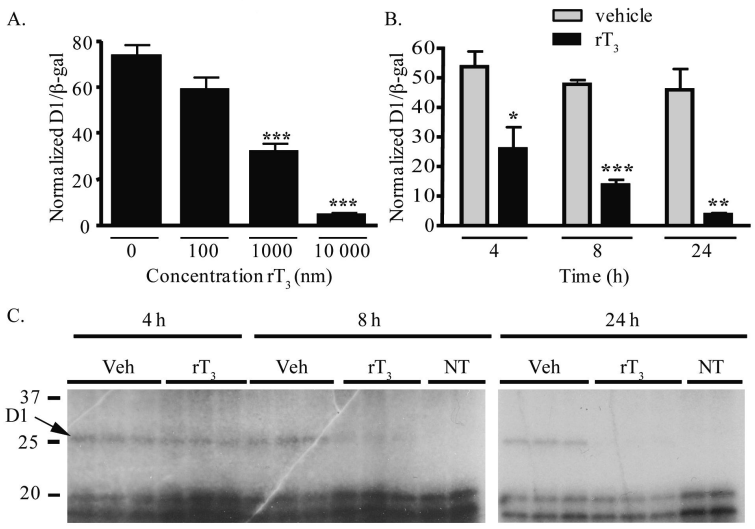

Fig. 4.

D1 activity is inactivated by rT3 exposure prior to a decrease in D1 protein in a transfected cell system. (A) HEK293 cells were transfected with vectors expressing human D1 and β-galactosidase. Cells were treated with the indicated concentration of rT3 for 20 hours, then D1 activity measured in sonicates in the presence of 10 mM DTT and is shown normalized by β-galactosidase activity. (B) HEK293 cells were transfected as in (A) and were treated with either vehicle or 10 μM rT3 for the indicated time. D1 activity was measured as in (A). The mean ± SEM of 3 wells/group are shown for (A) and (B), with similar results being obtained in 2-3 experiments. For (A) ***=$p<0.001$ by one-way ANOVA when compared to the vehicle control. For (B) the effect of rT3 treatment was significant ($p<0.001$) over time by two-way ANOVA, followed by student's t-testing between the vehicle and rT3 treated groups at each time with ***=$p<0.001$, **=$p<0.001$, *=$p<0.05$. (C) HEK293 cells were transfected as in (A) and were labeled with ^{75}Se for 19 h prior to treatment with either vehicle or 10 μM rT3 for the indicated time. Cell lysates were run on a SDS-PAGE gel, which was then dried and used for autoradiography. All samples were collected within the same experiment and were run on two gels. Lysates from non-transfected (NT) cells are shown on the far right, and the band corresponding to D1 is indicated on the left by an arrow.

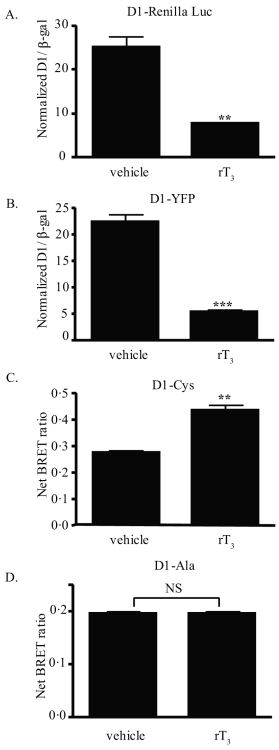

Fig. 5.

rT3 treatment increases BRET signal between D1-dimers. (A, B) HEK293 cells were transfected with the D1-Renilla Luc (A) or D1-YFP (B) expression plasmids as indicated, along with a β-galactosidase expression vector. Cells were treated with 3 μM rT3 as indicated for 20 hours, and D1 activity in sonicates was measured in the presence of 10 mM DTT. The mean ± SEM is shown of 3 wells/group, with similar results being obtained in 2 experiments. (C) BRET between D1-Renilla Luc and D1-YFP with cysteine substituted in the active center or (D) alanine substituted in the active center was measured after treatment with 10 μM rT3 for 1 hour as indicated. The mean ± SEM of triplicate determinations are shown for each treatment group, with similar results being obtained in 2-3 independent experiments. ***=p<0.001, **=p<0.001, by student's unpaired t-test.