Thyroid: biological actions of ‘nonclassical’ thyroid hormones

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Abstract

Thyroid hormones (THs) are produced by the thyroid gland and converted in peripheral organs by deiodinases. THs regulate cell functions through two distinct mechanisms: genomic (nuclear) and nongenomic (non-nuclear). Many TH effects are mediated by the genomic pathway – a mechanism that requires TH activation of nuclear thyroid hormone receptors. The overall nongenomic processes, emerging as important accessory mechanisms in TH actions, have been observed at the plasma membrane, in the cytoplasm and cytoskeleton, and in organelles. Some products of peripheral TH metabolism (besides triiodo-L-thyronine), now termed ‘nonclassical THs’, were previously considered as inactive breakdown products. However, several reports have recently shown that they may have relevant biological effects. The recent accumulation of knowledge on how classical and nonclassical THs modulate the activity of membrane receptors, components of the mitochondrial respiratory chain, kinases and deacetylases, opened the door to the discovery of new pathways through which they act. We reviewed the current state-of-the-art on the actions of the nonclassical THs, discussing the role that these endogenous TH metabolites may have in the modulation of thyroid-related effects in organisms with differing complexity, ranging from nonmammals to humans.

Key Words

• thyroid hormone
• nongenomic effects
• thyroid hormone metabolism
• thyroid hormone receptors

Introduction

General notions

The thyroid gland produces two main iodothyronines: tetraiodo-L-thyronine (T4) and triiodo-L-thyronine (T3). In humans, T4 is synthesized entirely within the thyroid and acts as a pro-hormone to generate T3. Only 20% of the T3 in circulation is secreted directly by the gland itself. The remaining T3 derives from the peripheral monodeiodination of T4. Deiodinase activity regulates the local and systemic availability of T3 and other iodothyronines. Thyroid hormone (TH) deiodination is mediated by three selenoenzymes: type 1 deiodinase (D1), preferentially expressed in the liver and also expressed in the kidney, thyroid, and pituitary; D2, present in the CNS, anterior pituitary, brown adipose tissue, and placenta; and D3 in the CNS, placenta, skin, and fetal tissue. For further details on deiodinases, the reader is referred to Bianco (2011), Maia et al. (2011), Orozco et al. (2012) and Luongo et al. (2013).

Other biochemical pathways are involved in TH metabolism in addition to deiodination. Conjugation of phenolic hydroxyl groups with sulfate or glucuronic acid...
involves different signaling transduction pathways. These effects are mediated by nongenomic actions. The overall nongenomic processes are poorly understood but emerge as important accessory mechanisms in TH actions and have been observed at the plasma membrane, in the cytoplasm and cytoskeleton, and in organelles (Wrutniak-Cabello et al. 2001, Cheng et al. 2010). Membrane receptors, consisting of specific integrin alpha V beta 3 (αVβ3) receptors, have been identified (Bergh et al. 2005). THs on the cell surface trigger the serine–threonine kinase (MPK/ERK) pathway via the integrin receptor (Bergh et al. 2005, Cody et al. 2007), initiating complex cellular events (Lin et al. 2009a,b). In the cytoplasm, THs activate PI3K and thereby downstream gene transcription of specific genes. T3 also activates PI3K from the integrin αVβ3 hormone receptor site (Lin et al. 2009b, Moeller & Broecker-Preuss 2011). Calcium is a second messenger regulated by THs through the modulation of a Ca2+-ATPase (Galo et al. 1981). Del Visco et al. (2012) showed that THs exert short-term nongenomic effects on intracellular calcium by modulating plasma membrane and mitochondrial pathways in rat pituitary GH3 cells. Furthermore, cellular actions involving Akt/protein kinase B (shown in human fibroblasts; Moeller et al. 2005) and AMP-activated protein kinase (AMPK) (in mice) (Ircher et al. 2008) are well known. De Lange et al. (2008) showed that in rat skeletal muscle, T3 stimulates FA and glucose metabolism through rapid activation of AMPK and Akt/protein kinase B signal transduction.

THs regulate mitochondrial activity and thus it may perhaps not be surprising that the mitochondria themselves are important target for THs. THs modulate mitochondrial activity through two ways: direct or indirect. The first requires the presence inside the organelles of specific binding sites for THs that play important physiological roles in regulation of the mitochondrial transcription apparatus (see for review, Cioffi et al. (2013)). One of these binding sites, termed p43, has been identified as a bona fide TR that binds to the D-loop region that contains the promoters of the mitochondrial genome (Wrutniak et al. 1995). By contrast, the indirect way acts through increased, nuclear TR-dependent transcription of factors that modulate the expression of mitochondrial genes (see for review, Cioffi et al. (2013)).

The nonclassical THs
Beside T3, nonclassical THs exist. In the present review, we summarize the highlights of their biological actions.

TH actions
THs regulate cell functions through two distinct mechanisms: genomic (nuclear) and nongenomic (non-nuclear). Most effects of TH are mediated by the genomic pathway—a mechanism that requires thyroid hormone activation of nuclear receptors (TRs). This leads to a conformational change allowing interaction with specific thyroid hormone responsive elements located on the promoters of THs target genes (Bassett et al. 2003, Moeller & Broecker-Preuss 2011, Tata 2013, Pascual & Aranda 2011), regulating transcription rate. TRs homodimerize or interact with other nuclear receptors such as the retinoic X receptor (Forman et al. 1992, Bogazzi et al. 1994). TRs belong to a large family of ligand-dependent transcription factors, which includes nuclear hormone receptors for vitamins, xenobiotics, and sex steroids (Weitzel & Alexander Iwen 2011). They are termed as TRα and TRβ and are encoded by two genes (α and β) located on two different chromosomes (Cheng 2000) that express differently in developing and adult tissues (Oetting & Yen 2007, Cheng et al. 2010). Highest Trα1 (Thra) expression is in the brain, with lower levels in the kidney, skeletal muscle, lungs, heart, and liver, whereas Trβ1 is expressed predominantly in the kidneys and liver, and at lower levels in the brain, heart, thyroid skeletal muscle, lungs, and spleen (Williams 2000). TRβ isoforms are involved in lipid metabolism (Pramfalk et al. 2011) by reducing serum lipids (Johansson et al. 2005, Angelin & Rudling 2010, Shoemaker et al. 2012). TRβ disruption in mice impairs fatty acid (FA) oxidation (Araki et al. 2009) even in the presence of TRα overexpression (Gullberg et al. 2000, 2002). TRβ agonists have approximately tenfold greater affinity for TRβ than TRα, with a marked effect on the liver and efficacy in lowering of cholesterol (Webb 2010, Ladenson 2011). Although T3 exerts many of its actions through canonical transcriptional regulation, an increasing amount of evidence shows that many of T3 effects are initiated outside the nucleus and
Tetrac and Triac

In humans, the amount of Triac produced by the liver and other tissues accounts for about 14% of T₃ metabolism (Siegrist-Kaiser & Burger 1994). Triac is weakly TRβ-selective, with a 1.5-fold affinity for TRβ (Schueler et al. 1990). Triac has been used to suppress thyroid-stimulating hormone (TSH) secretion in TH-resistant patients (Kunitake et al. 1989) and to increase metabolic rate in obese patients (Dumas et al. 1982). It has been shown to be more potent than T₃ as both a β-adrenergic stimulator of uncoupling protein 1 and inducer of lipoprotein lipase mRNA, D3 activity, and mRNA (Medina-Gomez et al. 2003). Triac inhibits expression and secretion of leptin in rat primary white and brown adipocytes with a potency similar to that of T₃ (Medina-Gomez et al. 2004). The use of Tetrac as a potential substitute for T₄ has been studied in the treatment of myxedema and for its ameliorating effect on peripheral lipid metabolism in humans. The effects are similar to those of T₄, but require higher dosing (Lerman 1956). Tetrac is currently used in the clinic for the treatment of TH resistance (Anzai et al. 2012). Therapeutic doses of Triac to treat pituitary and thyroid disorders exceed those required for T₄ and T₃ (Sherman & Ladenson 1992, Bracco et al. 1993), a property attributed to its short half-life in humans and rodents (Pittman et al. 1980, Moreno et al. 1994). Classic THs are transported within the cell by TH transporters (Visser 2013). Tetrac does not seem to depend on active transport, at least by the most abundant transporter MCT8. Tetrac can replace T₃ to restore normal fetal mouse brain development in MCT8-null mice (Horn et al. 2013).

Thyronamines

The structures of the thyronamines (TAMs), a novel class of endogenous thyroid-signaling molecules, differ from T₄ and deiodinated TH derivatives by the absence of a carboxylate group on the alanine side chain. 3-Iodothyronamine (T1AM) and T0AM have been detected in vivo (Scanlan et al. 2004, DeBarber et al. 2008) in the serum of rodents and humans (Saba et al. 2010, Hoefig et al. 2011), in rat liver, brain, and heart (Chiellini et al. 2007, Saba et al. 2010). Data from Piehl et al. (2008) present a role for deiodinases in TAM biosynthesis, defining biosynthetic pathways for T1AM and T0AM with T₄ as a pro-hormone. Seemingly in contrast, a recently developed method to detect T1AM and T0AM in tissues and plasma (Ackermans et al. 2010) failed to reproduce the above data. Using rats treated with (13)C-labeled T₄, the authors could detect in vivo conversion of T₄ to T₃ but not to T1AM in plasma or brain samples, neither any endogenous T1AM nor T0AM was detected in the plasma from rats and plasma and in thyroid tissue from humans. Indeed, iodothyronine decarboxylation to iodothyronamines has not been demonstrated directly, and the aromatic amino acid decarboxylase was shown to be unable to catalyze iodothyronine decarboxylation (Hoefig et al. 2012). In line with this, data from Hackenmueller et al. (2012) suggest that T1AM is not an extrathyroidal metabolite of T₄, yet is produced within the thyroid by a process that requires a sodium–iodide symporter and thyroperoxidase, the same biosynthetic factors necessary for T₄ synthesis. These data shed new light on the pathways potentially involved in T1AM production and imply that the enzymatic conversion of iodothyronine to iodothyronamine is not simple. Steady-state physiological T1AM serum concentrations are similar to those of T₃, and tissue concentrations of its metabolite, T0AM, exceed T₄ and T₃ metabolites by two- and 20-fold respectively (Hart et al. 2006, Chiellini et al. 2007). Physiological receptor(s) of TAMs remain to be identified. In TR receptor binding/gene activation assays, T1AM showed no affinity for TRβ and TRz, and inability to modulate nuclear TR-mediated transactivation (Chiellini et al. 1998). Studies surrounding TAM association with other receptors concluded that neither T0AM nor 3-T1AM activated Gαs-coupled dopamine D1 and β2 adrenergic receptors (Scanlan et al. 2004). T1AM, however, was found to be a potent agonist of trace amine-associated receptor 1 (TAAR1), an orphan G protein-coupled receptor (Zucchi et al. 2006). Rat and mouse TAAR1 are activated by T1AM, with EC50 values of 14 and 112 nM respectively. The T1AM ligand pharmacophore that activates TAAR1 was later characterized (Hart et al. 2006, Tan et al. 2007, 2008, Snead et al. 2008). T1AM reduces activation of the proto-oncogene c-fos (Manni et al. 2012). Ianculescu et al. (2009) reported that the cellular uptake of T1AM occurs via specific, saturable, and inhibitable transport mechanisms that are sodium and chloride independent, pH dependent, TAM specific, and do not involve candidate transporters of monoamines, organic cations, or THs. By a novel RNAi screening method, eight transporters of interest were identified. Knockdown resulted in T1AM transport in HeLa cells, but the physiological role of these transporters remains unknown. Studies using COS-1 cells transfected with multispecific OATPs, 1A2, 1B3, and 1C1, and the specific TH transporters, MCT8 and MCT10, proved that T1AM differentially inhibits T₃ and T₄ cellular uptake by these transporters (Ianculescu et al. 2010). Notably, T1AM
also inhibits both T₃ and T₄ uptake via MCT8, the most specific TH transporter. T1AM has no effect on TH transport by OATP1B3 and MCT10.

In mice, Scanlan et al. (2004) showed that a single i.p. injection of T1AM rapidly induced an ~10 °C drop in body temperature that peaked 1 h after injection and dose dependently disappeared after 4–6 h. The same authors further showed that T1AM reduction on cardiac performance was a direct effect and independent of T1AM-induced hypothermia. In a rat working heart preparation held at 37 °C, introduction of T1AM into the perfusion buffer resulted in large and immediate decreases in both heart rate and systolic aortic pressure. Additional studies on the heart have further supported direct actions of T1AM on this organ (Chiellini et al. 2007, Frascarelli et al. 2008). A single i.p. dose of T1AM dramatically switched fuel utilization away from carbohydrates and toward lipids (Braulke et al. 2008). Siberian hamsters (Phodopus sungorus), a hibernating rodent species, and mice completely shifted their respiratory quotients (RQ) from a normal, mixed carbohydrate and lipid value (0.90 for hamsters and 0.83 for mice) to a complete and persistent lipid-related RQ value of ~0.7 with elevated urine ketone content. The RQ effect (4.5 h after injection) lagged behind hypothermia, bradycardia, or hyperglycemia (1 h after injection). I.v. infusion with a low T1AM dose (0.5 mg/kg) into nonfasted naive rats rapidly increased endogenous glucose production and plasma glucose, plasma glucagon, and corticosterone, but did not affect plasma insulin (Klieverik et al. 2009). Contrastingly, in i.v.c. injected (130 ng/100 g body weight (BW)) short-term fasted male mice (Manni et al. 2012), T1AM failed to ameliorate lipid profiles. It is known to possess a central effect, namely hypophagia, as well as peripheral effects of raised plasma glucose levels and reduced peripheral insulin sensitivity (the latter being also seen after i.p. injection (Braulke et al. 2008, Klieverik et al. 2009)), accompanied by pancreatic insulin production. Plasma free T₃ (fT₃) levels were also lowered. Nonfasted, drug-naïve rats (Klieverik et al. 2009) treated with T1AM (100 µg/kg) acutely increased endogenous glucose production and hyperglucagonemia, while in contrast to the effect in fasted mice (Manni et al. 2012) plasma insulin decreased. T0AM had a similar effect that was less profound (Klieverik et al. 2009). Interestingly, T1AM injection in mice resulted in 12% of the injected dose in the plasma, highlighting its systemic bioavailability (Manni et al. 2012). Inhibition of T1AM conversion by pretreatment with a mitochondrial amine oxidase inhibitor, clorgyline (250 µg/100 g BW), prominently increased T1AM serum levels, but prevented the hyperglycemia and reduction of fT₃ levels. This led the authors to indicate that a metabolite of T1AM causes these adverse effects. Central effects of T1AM administration also included amelioration of memory and reduction in pain threshold (Manni et al. 2013). T1AM’s enhancing effect on learning renders this compound useful in the treatment of neurodegenerative diseases.

3,3′,5′-Triiodo-L-thyronine

3,3′,5′-T₃ (reverse T₃, abbreviated as rT₃), a product of 5-deiodination of T₄ by D1 and D3, is a potent initiator of actin polymerization in astrocytes. It portrays similar effectiveness to T₄ and much more than T₃ (Farwell et al. 2006). In hypothyroid rodents, neurons and astrocytes develop poor actin cytoskeletons that T₃ replacement cannot rescue. However, rT₃ initiates reappearance of filamentous actin within minutes without altering total actin mRNA or protein content (Farwell et al. 1990, Siegrist-Kaiser et al. 1990). This rT₃ property is attributed to TRΔx1, a native TR isofrom that lacks a nuclear localization signal and is present in the extranuclear compartment of astrocytes and neurons. This isofrom has the ligand affinity and specificity required for of actin polymerization by rT₃. A study of the astrocytes of the developing mouse cerebellum deprived of both TRs showed that TRΔx1 rescued the actin cytoskeleton’s response to rT₃ (Flamant & Samarut 2003). Thus, THs may require TR regions that are not necessarily canonical DNA-binding regions. rT₃ also inhibits free FA levels in chickens stimulated with dexamethasone or adrenaline (Bobek et al. 2002).

3,5-Diiodo-L-thyronine

Several studies have indicated 3,5-diiodo-L-thyronine (T₂), an endogenous metabolite of T₃, as a peripheral mediator of several TH metabolic effects. Although conversion of T₃ to T₂ has not been demonstrated in vitro, indirect evidence indicates that T₂ is indeed formed from T₃ in vivo through deiodination (Moreno et al. 2002). Serum concentrations of T₂ in humans are within the picomolar range (16 pM in healthy individuals to 50 pM maximum in individuals with nonthyroidal illness; Pinna et al. 1997). Rat intra-hepatic T₂ concentrations are 1.5 fmol/100 mg (Moreno et al. 2002). To date, results in hypothyroid rats suggest that T₂ has specific actions on resting metabolic rate (RMR) that are distinct from those of T₃: they are more rapid and not attenuated by actinomycin D (see Fig. 1A and B; Lanni et al. 1996).
Nonclassical thyroid hormones

T3 injection to euthyroid animals resulted in combined T3 and T2 patterns on RMR (see Fig. 1C). T3, propylthiouracil (inhibiting type 1 deiodinase and thyroid peroxidase activity), and iopanoic acid (inhibiting the activity of all three deiodinases) combined treatment showed a long-term induction of RMR. T2 is thus predominantly responsible for short-term induction of RMR (Fig. 1A; Moreno et al. 2002). The metabolic effects of T2 also underlined the ability of this hormone to improve survival of hypothyroid rats to cold with persistent increased energy expenditure (Lanni et al. 1998). Furthermore, upon T3 administration to hypothyroid rats, the RMR and FA oxidation increases in the muscle mitochondria are accompanied by mitochondrial translocation of the FA transporter FAT/CD36, ensuring effective increases in metabolic rate (Lombardi et al. 2012). Several data support the ability of T2 to stimulate mitochondrial activities in a very rapid manner (Lanni et al. 1992, 1993, 1994, Goglia 2005, Cavallo et al. 2011), excluding short-term genomic action of T2. Chronic treatment of hypothyroid rats with T2 revealed a different picture: T2 upregulated protein levels of ATP synthase subunits (alpha, beta, F(o)I-PVP, and OSCP; Mangiullo et al. 2010). Increase in β-subunit mRNA accumulation suggested indirect transcriptional regulation by T2 through activation of the transcription factor, GA-binding protein/nuclear respiratory factor-2 (Mangiullo et al. 2010). Moreover, D1 activity in the rat anterior pituitary has been shown to be increased transiently after a single injection of T2, while in a reaggregate culture of anterior pituitary, T2 has been demonstrated to stimulate D1 at 24 h after its application, dose dependently (Baut et al. 1997).

T2, as a nonclassical TH, is able to prevent BW gain when administered i.p. to rats fed a high-fat diet without inducing T3-related undesirable side effects (tachycardia, cardiac hyperplasia, and decreased TSH levels), at least at the administered dose (25 µg/100 g BW for 4 weeks) (Lanni et al. 2005, De Lange et al. 2011, Moreno et al. 2011). At this dose, by almost doubling hepatic FA oxidation rate, T2 efficiently prevented HFD-induced i) hepatic fat accumulation, ii) insulin resistance, and iii) increase in serum triglycerides (TGs) and cholesterol levels (Lanni et al. 2005). T2 stimulated mitochondrial uncoupling, decreased ATP synthesis, and increased fat burning, thus counteracting obesity (Lanni et al. 2005).

An important consequence of the above-described effects of T2 included increased skeletal muscle insulin sensitivity, due to an increased response to insulin of Akt/PKB phosphorylation, and sarcolemmal glucose transporter 4 accumulation (Moreno et al. 2011). Importantly, T2 also induced biochemical and structural shifts...
toward glycolytic myofibers (Moreno et al. 2011). For an overview of T2’s effects on metabolism, see Fig. 2. Proteomic analysis of intracellular and mitochondrial proteins (Silvestri et al. 2010) revealed that mitochondria were the principal targets of T2’s normalizing effects on changes induced by high-fat diet (HFD). BN-page analysis revealed that T2 partially restored individual OXPHOS complex (predominantly complexes I and II) levels induced by HFD. Additionally, T2 augmented the activities of respiratory complexes, with respect to both HFD (except complex V) and N animals (except complex IV). In rats pre-fed a HFD, subsequent T2 administration reduced hepatic fat and hyperlipidemia via the same biochemical pathways described previously (Mollica et al. 2009).

T2 carries out its antilipemic effects via activation of two important factors involved in lipid metabolism: AMPK and nuclear deacetylase sirtuin 1 (SIRT1). AMPK is a known sensor of cellular ATP levels (Ruderman et al. 2013), and SIRT1 regulates metabolic balance in response to increases in cellular NAD⁺:NADH ratios. It has recently been identified to be a crucial target abating, diet-induced obesity in rodents (Rodgers et al. 2005, Lagouge et al. 2006, Gerhart-Hines et al. 2007). After simultaneous administration to rats receiving a high-fat diet, rapid induction of hepatic FA oxidation by T2 (within 6 h) was concomitant with hepatic activation of SIRT1, an activity persisting over time. Increased phosphorylation of AMPK was observed after 4 weeks of treatment (De Lange et al. 2011).

Figure 2
Thyroid synthesis of thyroid hormones (THs) and administration effects of nonclassical THs. Question mark, thyroid synthesis of T2 and T1AM is currently still under debate. Peripheral synthesis of nonclassical THs by deiodinase activity (see text) is not indicated. Administration of nonclassical THs (for explanations of treatments, see text) indicated in color. White, Triac incubation in rat primary cultures; yellow, i.p. administration of T2 in rats on a high-fat diet; green, i.p. or i.v. administration of T1AM in mice; grey, i.v. administration of T1AM in mice; red, i.c.v. administration of T1AM to fasted mice; blue, i.c.v. administration of T1AM to mice; and purple, rT3 administration to mice. Arrows pointing upward, upregulation; arrows pointing downward, downregulation; double horizontal arrows, no change; and absence of arrows, no effect reported to date.
Induction of SIRT1 led to deacetylation of peroxisome proliferator-activated receptor γ coactivator-1α and sterol receptor element binding protein-1c (SREBP-1c), associated with induction and reduction of expression of genes involved in FA oxidation and lipogenesis respectively (De Lange et al. 2011). These findings provide a clue to explain T2’s effectiveness in lowering hepatic fat accumulation and counteracting insulin resistance with respect to T3. In a similar system, T3 has increased hepatic lipogenesis (Cable et al. 2009).

A second clue indicating that T2 has contrasting effects on hepatic lipogenesis involving SREBP-1c was found in vitro. T3 increases an active precursor of SREBP-1c in HepG2 cells without modulating SREBP-1c transcription (Gnoni et al. 2012). T2, however, blocks proteolytic cleavage and thus activation of SREBP-1c (Rochira et al. 2013) independent of transcription. Consequently, FA synthase expression reduced. The resulting inhibitory effect of T2 on lipogenesis is concordant with in vivo findings (De Lange et al. 2011) and mechanistically complementary to SIRT1-dependent deacetylation of SREBP-1c (De Lange et al. 2011). The liver is not the only organ in which T2 activates SIRT1: T2 has been shown to act through SIRT1 activation in the kidney (Shang et al. 2013). Treatment with T2 prevented diabetic nephropathy (DN) in a diabetic rat model via SIRT1-dependent deacetylation of SREBP-1c (De Lange et al. 2011). 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Additionally, total serum cholesterol levels were lowered, and no side effects were recorded (Antonelli et al. 2011). A mechanism by which T2 reduces cholesterol in the serum (Lanni et al. 2005, Antonelli et al. 2011, De Lange et al. 2011) was recently called into question. T2’s LDL-lowering effects are independent of the LDL receptor (Goldberg et al. 2012), as determined by feeding a western type diet to LDL receptor-deficient mice (Ldlr−/−) and treating with T2. The diet was chosen because dietary absorption of cholesterol and TG drives hepatic apoB production, especially in Ldlr−/− mice. These mice develop much higher levels of cholesterol and atherosclerosis. T2 had no effect on TG levels, probably due to increased lipolysis, but led to marked reductions in liver apoB and circulating apoB48 and apoB100 (Goldberg et al. 2012).

To study whether the lipid-lowering effects of T2 were directly acting on the liver, or if they were secondary to changes in endocrine or metabolic pathways, primary rat hepatocytes, overloaded with lipids (to obtain ‘fatty hepatocytes’) and treated with T2 (10−5 M) have been employed (Grasselli et al. 2011a). This experimental setup demonstrated that T2 reversed the effects induced by lipid overload in these cells, thus supporting a direct effect of T2. Moreover, rat hepatoma (FAO) cells defective for functional TRs were used to answer whether T2-mediated lowering of hepatic lipid profiles even requires TR action (Grasselli et al. 2011b). Exposure to pharmacological doses of T2 (10−5 M) for 24 h reversed the effects induced by FAs and increased mitochondrial uncoupling, thus indicating that the actions of T2 in these cells are independent of transcriptionally functional TRs.

Intracellular action of T2 also is described in avian cells during fetal development and cell differentiation (Incerpi et al. 2002). T2 regulates DNA synthesis, cell-cycle proteins (Alisi et al. 2004), and several membrane-associated transport systems, whose activity is related to cell proliferation (Incerpi et al. 2002, 2005). T2’s effect on the Na+/H+ exchanger was identified for 14-day- and 19-day-old cells, whereas the effect on amino acid transport was present at late stages of embryo development. Both transport systems were activated through a signal transduction pathway involving the PKC, MAPK, and PI3K pathways (Incerpi et al. 2002). Moreover, T2 exerts a short-term inhibitory effect on the Na+/K+ -ATPase, the magnitude of which strongly correlates to the developmental age of the isolated cells (Scapin et al. 2009). The Na+/K+ -ATPase inhibition is mediated through the activation of PKA, PKC, and PI3K (Scapin et al. 2009). Signal transduction pathways contributing to the modulation of the sodium pump by T2 are involved in the control exerted on cell proliferation.

Recently, it has been shown that T2 exerts short-term effects on intracellular calcium concentrations and NO release by modulating plasma membrane and mitochondrial pathways in pituitary GH3 cells (Del Viscovo et al. 2012). In particular, T2 facilitates physiological Ca2+...
**Table 1** Involvement of TRs or alternative receptors in non-classical thyroid hormone action

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<thead>
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<th>Non-classical thyroid hormone</th>
<th>Affinity for/transactivation through TRα or TRβ</th>
<th>Affinity for other receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triac</td>
<td>High (Schueler et al. 1990)</td>
<td>None yet identified</td>
</tr>
<tr>
<td>T1AM</td>
<td>Absent (Chiellini et al. 1998)</td>
<td>High (trace amine-associated receptor 1 (TAAR1); Zucchi et al. 2006)</td>
</tr>
<tr>
<td>rT₃</td>
<td>Highᵃ (TRα₂1 (native TR isofrom); Flamant &amp; Samarut 2003)</td>
<td>None yet identified</td>
</tr>
<tr>
<td>T₂</td>
<td>Weak (human TRα; Cioffi et al. 2010)</td>
<td>None yet identified</td>
</tr>
<tr>
<td></td>
<td>Weak (human TRβ; Ball et al. 1997, Cioffi et al. 2010, De Lange et al. 2011, Mendoza et al. 2013)</td>
<td>None yet identified</td>
</tr>
<tr>
<td></td>
<td>High (short TRβ) of tilapia fish; Mendoza et al. 2013, Navarrete-Ramirez et al. 2014)</td>
<td>None yet identified</td>
</tr>
</tbody>
</table>

*Nongenomic action.

eflux from mitochondria through activation of mt-NCX by interacting with different mitochondrial complexes (Del Viscovo et al. 2012).

T₂’s biological effects are not restricted to mammalian species. Indeed, in the goldfish *Carassius auratus*, stimulation of pyruvate-fueled liver and muscle mitochondrial respiration was observed 5 min after 0.3 nM T₂ incubation (Leary et al. 1996). Garcia et al. (2004) examined the effects of short-term T₄, T₃, and T₂ exposure (0.1 μM; 12 or 24 h) on D1 and D2 activities and mRNA in killifish (tilapia) liver and showed that these hormones decreased D2 activity, the effect of T₂ being relatively more rapid. T₂ also regulates thermal acclimation in zebra fish (*Danio rerio*) with an efficiency comparable to T₃ (Little et al. 2013). The conserved role for T₂ on regulating metabolic efficiency in many species validates the motion to learn more about biological actions of this nonclassical TH.

**Mechanism of action of T₂**

Questions surrounding the cellular-molecular mechanism of action of T₂ remain. Both TR- and non TR-mediated actions may be elicited by T₂. Ball et al. (1997) reported that T₂ exerted selective thyromimetic effects. In the same report, T₂ showed a 60 times weaker affinity for TRβ than T₃. Mendoza et al. (2013) reported that in teleosts, effects of T₂ may be mediated by an isoform of one of the two known TRβs, namely TRβ1 that contains a 9-amino-acid insert in its ligand-binding domain (long TRβ1), whereas T₃ binds preferentially to a short TRβ1 isoform lacking this insert. Moreover, the authors confirmed that T₂ has a weak affinity for human TRβ (about 40-fold less than T₃) and a similarly weak transactivation capacity compared with T₃ (Ball et al. 1997, Cioffi et al. 2010, De Lange et al. 2011, Mendoza et al. 2013). In tilapia, both T₃ and T₂ are important in growth, a process, however, mediated by different TRβ1 isoforms (Navarrete-Ramirez et al. 2014). An overview of TR or alternative receptor involvement in the action of nonclassical THs is shown in Table 1.

Non TR-mediated effects of T₂ are evident. It is known that T₂ specifically stimulates the activity of isolated cytochrome c oxidase (COX) from bovine heart mitochondria. T₃ barely stimulates COX. T₂ binding to COX induces conformational changes. Studies show specific binding of labeled T₂ to the subunit Va of COX from bovine heart. T₂, and to a small extent T₃, but not thyroxine and thyronine, abolished allosteric inhibition of ascorbate respiration of reconstituted COX by ATP. Inhibition is rescued by a monoclonal antibody to the subunit Va. (Goglia et al. 1994, Arnold et al. 1998). T₂ directly activates SIRT1 (De Lange et al. 2011), influencing downstream pathways and inducing benefits. Shang et al. (2013) showed that this interaction mitigates a DN by using the Sirtuin inhibitor Sirtinol.

**Conclusion and perspectives**

It is clear that the so-called ‘nonclassical THs’ can induce various biological actions. TH derivatives exert important actions on metabolic parameters and on growth. At the cellular–molecular level, several pathways are affected, the most intriguing of which are related to lipid metabolism and signaling pathways. Beneficial effects of these molecules require more considerations due to their potential to modulate human health.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.
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