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MINI REVIEW

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Novel thyroid hormones

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Abstract

The field of thyroid hormone signaling has grown more complex in recent years. In particular, it has been suggested that some thyroid hormone derivatives, tentatively named "novel thyroid hormones" or "active thyroid hormone metabolites", may act as independent chemical messengers. They include 3,5-diiodothyronine (T2), 3-iodothyronamine (T1AM), and several iodothyroacetic acids, i.e., 3,5,3',5'-thyroacetic acid (TA4), 3,5,3'-thyroacetic acid (TA3), and 3-thyroacetic acid (TA1). We summarize the present knowledge on these compounds, namely their biosynthetic pathways, endogenous levels, molecular targets, and the functional effects elicited in experimental preparations or intact animals after exogenous administration. Their physiological and pathophysiological role is discussed, and potential therapeutic applications are outlined. The requirements needed to qualify these substances as chemical messengers must still be validated, although promising evidence has been collected. At present, the best candidate to the role of independent chemical messenger appears to be T1AM, and its most interesting effects concern metabolism and brain function. The responses elicited in experimental animals have suggested potential therapeutic applications. TA3 has an established role in thyroid hormone resistance syndromes, and is under investigation in Allen–Herndon–Dudley syndrome. Other potential targets are represented by obesity and dyslipidemia (for T2 and T1AM); dementia and degenerative brain disease (for T1AM and TA1); cancer (for T1AM and TA4). Another intriguing and unexplored question is the potential relevance of these metabolites in the clinical picture of hypothyroidism and in the response to replacement therapy.

Keywords Thyroid hormones · Thyronamines · Thyroacetic acids · Deiodinases

Introduction

Thyroid hormone, namely 3,5,3'-triiodothyronine (T3), and its precursor thyroxine (T4) undergo a complex metabolism in vivo. Enzymes acting on T4 and T3 include deiodinases, amine transferases, amine oxidases, decarboxylases, and several classes of conjugating enzymes, particularly sulfotransferases and UDP-glucuronosyl transferases [1]. According to the classical paradigm, T4 deiodination to T3 is an activating reaction, while all the other reactions represent inactivating processes, since they yield inactive compounds. This picture turned out to be simplistic. Some T4 or T3 derivatives can interact with nuclear thyroid hormone receptors (THR), or with other receptor types, and they produce significant functional effects when administered to experimental preparations or intact animals. It has been suggested that they should be regarded as chemical messengers, and the field of thyroid hormone signaling has been expanded to include the so-called "novel thyroid hormones" or "active thyroid hormone metabolites".

The alleged novel hormones include 3,5-didiodothyronine (T2); thyronamines, particularly 3-iodothyronamine (T1AM) and non-iodinated thyronamine (T0AM); thyroacetic acids, particularly 3,5,3',5'-thyroacetic acid (TA4), 3,5,3'-thyroacetic acid (TA3), and 3-thyroacetic acid (TA1) (Figs 1–3).

In this review, we summarize the present knowledge on these compounds, starting with their biosynthetic pathways, endogenous levels, and molecular targets. We then describe the functional effects elicited in experimental preparations or intact animals after exogenous administration. The latter have stimulated speculations about potential therapeutic applications, and have provided the basis to discuss the physiological and/or pathophysiological role of the novel

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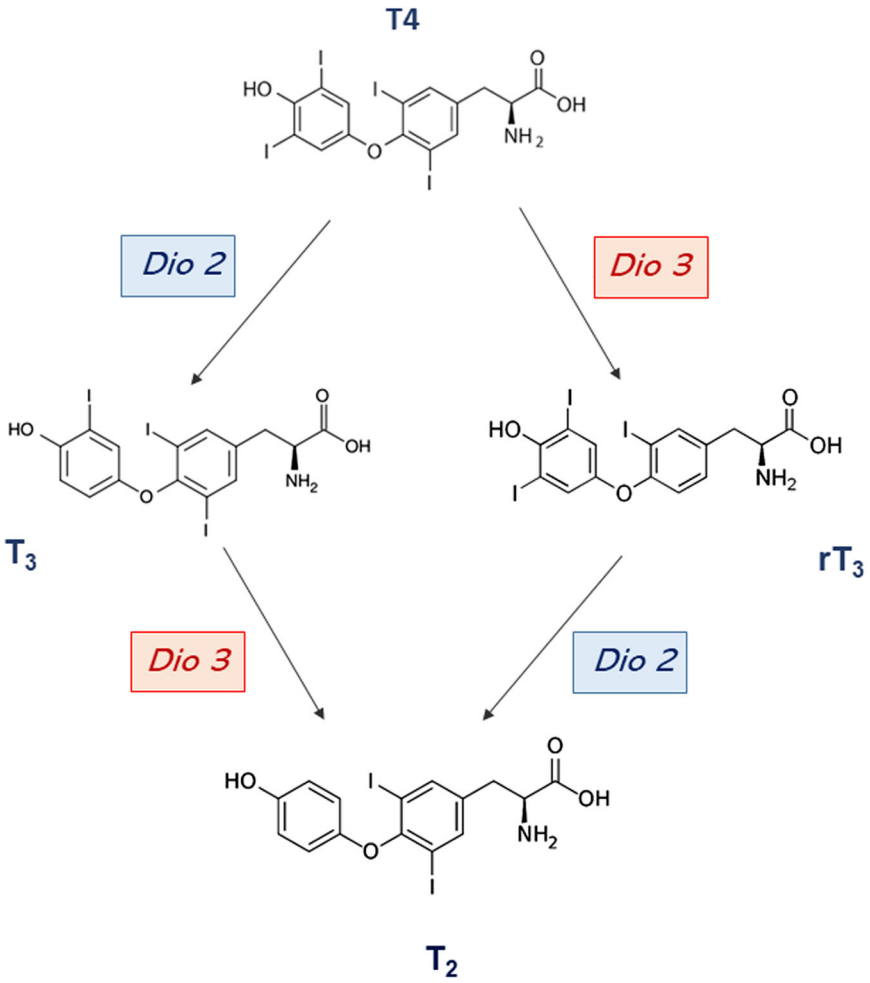

Fig. 1 Possible pathways of T2 production. Dio2, type II deiodinase; Dio3, type 3 deiodinase. Note that type I deiodinase can catalyze iodine removal from either aromatic ring, but its affinity is much lower than Dio2/Dio3 affinity. See the text and ref. [2] for further details

thyroid hormones. Unfortunately, our present knowledge is limited, and some crucial details are still missing. However, the general picture suggests that a deeper attention to these compounds may open interesting perspectives.

3,5-diiodothyronine (T2)

T2 can be obtained from T3 through deiodination of the outer (phenolic) aromatic ring (Fig. 1). This reaction is catalyzed by type I or type II deiodinase, which are expressed in many organs [2], and since the 70's several investigations have detected endogenous T2 in blood and the liver, both in experimental animals and in humans [3].

The physiological range of serum T2 has not been definitely established (Table 1). The initial immunoassays provided the variable results. More recently, Lehmphul et al. validated a novel immunoassay, which has been used in large clinical series [4, 5], and the median concentration in normal subjects was 290 pmol/L [4]. Liquid chromatography coupled to mass spectrometry (LC–MS), usually regarded as the gold standard in clinical chemistry, has also been used. In the first reports, the T2 isomer 3,3'-didiodothyronine (3.3'-T2) was identified, at very low concentration (<1 pmol/l), but T2 could not be assayed. Quite recently, we could detect endogenous T2 in a small sample of normal human subjects, with an average concentration of 78 pmol/l, versus 253 pmol/l for 3,3'-T2 [6]. Similar levels have been reported in frog serum [7]. Tissue data, are available for the human placenta [8], were T2 averaged 160–260 pg/g, i.e., about 300–450 pmol/Kg.

The differences between LC–MS and immunoassay results might be accounted for by protein binding. While it is well known that over 99% of serum T3, and probably of serum T1AM, is protein bound (see below), no direct data are available for T2. The transporters responsible for T2 cellular uptake are also poorly characterized.

T2 is a THR agonist, but it is 50–1000-fold less potent than T3 at THR beta [9], and it is even less active at THR alpha [10]. Rapid, non-genomic, actions of T2 on mitochondrial function and cellular metabolism have also been reported [11, 12]. Specific binding sites have been described in the respiratory chain complex IV (subunit Va). Additional molecular targets may be represented by

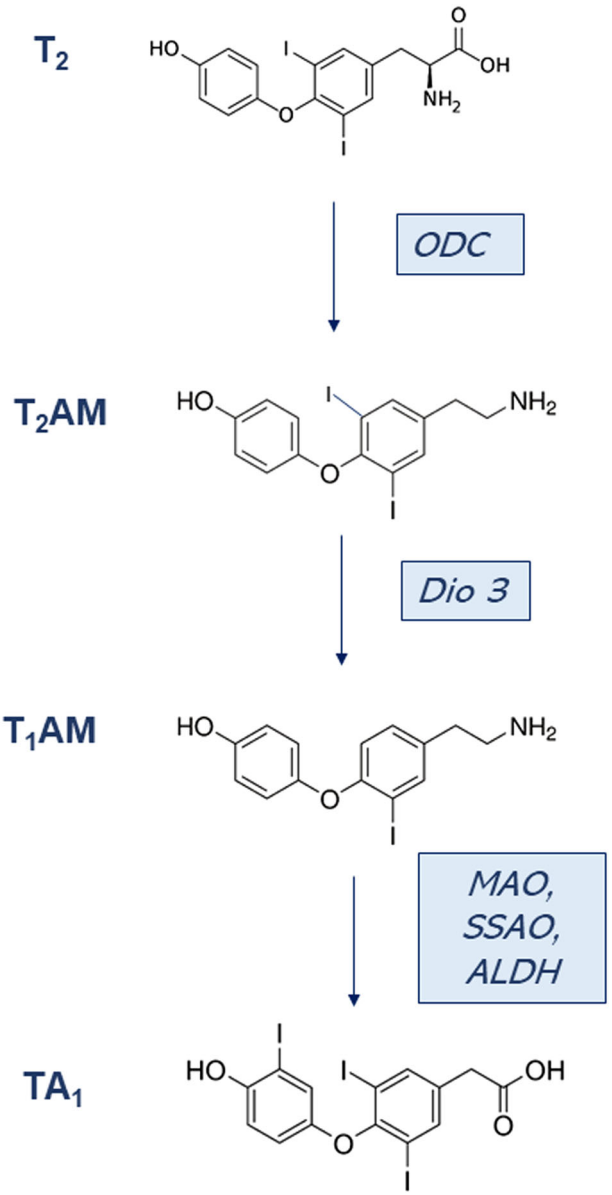

Fig. 2 The hypothetical pathway of T1AM and TA1 production. ALDH aldehyde dehydrogenases, Dio3 type 3 deiodinase, MAO monoamine oxidases, ODC ornithine decarboxylase, SSAO semicarbazide-sensitive amine oxidases. See the text and refs [39, 40] for further details

AMP-activated protein kinase (AMPK), a major regulator of energy metabolism, and by histone deacetylase sirtuin 1 (SIRT1). SIRT1 induction could be correlated with the repression of crucial genes of lipogenesis, via PPARγ coactivator-1a and sterol receptor element-binding protein-1c (SREBP-1c). However, the affinity of these putative binding sites, and/or the identity of the underlying signaling pathways, has not been determined.

The administration of exogenous T2 to experimental animals stimulated lipolysis and oxidative lipid metabolism, leading to decreased body weight in rodent models of diet-induced obesity. Reduction in serum cholesterol and in serum and liver triglycerides were also observed. Moreover, T2 was shown to reduce hepatic steatosis in animal models of nonalcoholic fatty liver disease (functional responses are reviewed by Senese et al. and Goglia [12, 13]). While originally attributed to direct mitochondrial effects, these responses were associated with significant changes in gene expression, which were not identical to those induced by T3, suggesting specific genomic effects [14, 15].

It is still controversial whether these metabolic responses can be dissociated from other thyromimetic effects, particularly potentially detrimental cardiac effects. While in rats 0.25–0.75 μg/g T2 did not affect cardiac function [13, 14], in mice evidence of cardiac hypertrophy and pituitary suppression was observed at similar or higher dosages (0.25 or 2.5 μg/g) [15]. It is still unclear whether the discordant results can be accounted for by differences in species and dosage, and further investigations are needed to clarify this crucial issue.

The physiological role of endogenous T2 is unclear, since endogenous levels seem too low to produce significant functional effects, although it should be stressed that very few data are available for tissue T2 concentration, and that the affinity for mitochondrial targets has not been established. A slight increase in serum T2 has been reported in patients with nonthyroidal illness syndrome [16], but the implications of these findings are unclear. While T2 is believed to be produced from endogenous T3, Lehmphul et al. failed to detect significant changes in serum T2 in hypothyroid or hyperthyroid patients [4]. Consistently, no significant correlation between serum T2 and T3 or T4 was reported by Lorenzini et al. [6].

On the basis of the animal investigations described above, T2 has been proposed as a potential therapeutic agent in obesity and/or dyslipidemia. The literature includes a single report of a pilot investigation, in which two human volunteers received T2 (300 μg/day) for 3 weeks [17]. Increased resting metabolic rate and decrease body weight were observed, without any change in cardiac parameters. A synthetic T2 analog (TRC150094) has been tested in a small group of hypercholesterolemic patients, but no change in lipid profile was detected at the dosage of 50 mg once daily [18]. Before further clinical investigations are planned, the issue of the ratio between therapeutic and toxic dosages of T2 should be better evaluated in experimental models.

Thyronamines

Thyronamines derive from thyronines through decarboxyation. So far only two thyronamines have been detected in tissues, namely T1AM and T0AM [19–21]. A biosynthetic pathway for T1AM has been identified by administering

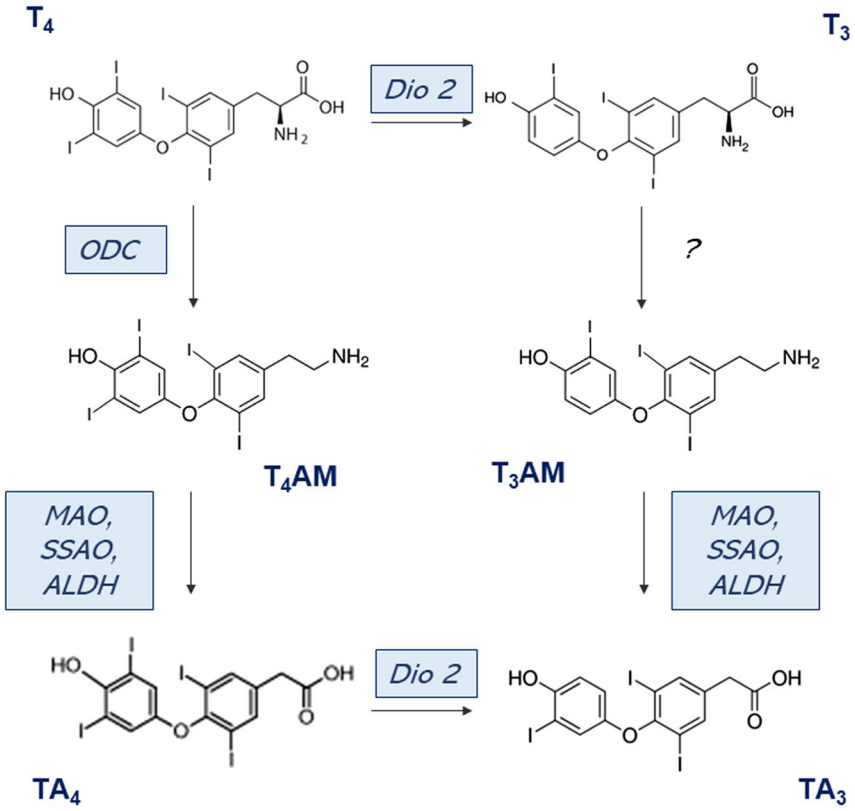

Fig. 3 The hypothetical pathway of TA4 and TA3 production. ALDH aldehyde dehydrogenases, Dio2 type 2 deiodinase, MAO monoamine oxidases, ODC ornithine decarboxylase, SSAO semicarbazide-sensitive amine oxidases. See the text for further details and alternative possibilities

exogenous T3 or T4 to a rat gut preparation [22]. It involves deiodination to T2, followed by decarboxylation to T2AM (catalyzed by ornithine decarboxylase), and further deiodination to T1AM (catalyzed by type III deiodinase) (Fig. 2). It is still unclear whether this is the only biosynthetic pathway and/or whether T1AM synthesis from T3 may occur in other tissues. In particular, minimum T1AM production after administration of exogenous T3 has been reported in cardiomyoblasts [21]. T1AM is a substrate for many different enzymes, including amine oxidases, type III deiodinase, N-acetyl-transferases, sulfotransferases, and UDP-glucuronosyl transferases, yielding a variety of metabolites (reviewed by Scanlan et al. and Kohrle et al. [19, 23]). In most tissues, the major metabolite of exogenous T1AM is TA1, while in blood comparable levels of O-sulfonate-T1AM and T1AM-glucuronide are observed.

T1AM can be taken up and concentrated by tissues, although its transporters have not been clearly identified. Proteins belonging to the SLC family were allegedly involved in sodium-independent and pH-dependent T1AM uptake [24], while in the heart T1AM uptake appeared to be sodium dependent [21]. Since T1AM can bind LDL lipoproteins (see below), LDL uptake might contribute to T1AM cellular uptake. In any case, T1AM has a widespread distribution after systemic administration, reaching virtually every organ [25]. Most exogenous T1AM is recovered in the liver and the gastrointestinal tract, where it undergoes biliary excretion, and long-term (24 h) storage occurs in the muscle, liver, and adipose tissue.

Endogenous T1AM is present in virtually every rodent tissue, and T0AM was detected in the liver [21]. T1AM tissue levels are on the order of a few pmol/g (nmol/Kg) (reviewed by Hoefig et al. and Kohrle et al. [20, 23]), comparable or even superior with tissue T3/T4 levels. Tissue assays were based on LC–MS. When the same technique was used in rat or human blood, T1AM concentration turned out to be lower (e.g., 0.2 nmol/l) [26] and often fell below the limit of detection of the assay [27]. By contrast, an immunoassay has been developed [28], which yielded human serum T1AM concentration in the range of 4–66 nmol/l [28–30] (Table 1). These discrepancies have been attributed to extensive protein binding, speculating that less than 1% of circulating T1AM is free, and that protein-bound T1AM is lost during the extraction procedures needed for the LC–MS assay. However, recent findings suggest the existence of additional technical pitfalls, including potential adduct formation in serum [31].

Thyronamines do not interact with THR, but they are high-affinity agonists of trace amine-associated receptor 1 (TAAR1), a membrane G protein-coupled receptor [19]. T1AM affinity for rat TAAR1 lies in the mid nanomolar range, while T0AM affinity is about tenfold lower. In other species, including human, EC50's are slightly higher and may reach the low micromolar range [32]. However,

Table 1 Serum concentrations of "novel thyroid hormones" in human

Metabolite	Concentration pmol/L	Technique	References
3,5-didiodothyronine (T_2)	78	LC–MS	[31]
	200	IA	[4]
3-iodothyronamine (T_1AM)	200	LC–MS	[26]
	5000–66000	IA	[28–30]
Thyronamine (T_0AM)	Not detected	–	
3,5,3',5'-tetraiodothyroacetic acid (TA_4)	712	IA	[53]
3,5,3'-triiodothyroacetic acid (TA_3)	42–244	IA	[52]
3'-iodothyroacetic acid (TA_1)	Not detected	–	

IA immunoassay, *LC–MS/MS* liquid chromatography coupled to mass spectrometry

additional molecular targets have been reported, including other TAAR subtypes, alpha2 adrenergic receptors, transient receptor potential channels, and ApoB-100, a component of VLDL and LDL lipoproteins, the latter probably accounting for high-affinity protein binding in serum (reviewed by Hoefig et al. and Kohrle et al. [20, 23]).

This picture is not surprising. The discovery of T1AM was based on the concept that amines derived from aromatic aminoacids usually act as chemical messengers. As a matter of fact, most classical aromatic amines (e.g., catecholamines, histamine, and serotonin) are multi-target ligands, and the same appears to be true for T1AM.

Many functional responses have been elicited in intact rodents or in experimental preparations after the administration of exogenous T1AM. The initial observations focused on hypothermia and cardiac effects, namely reduced heart rate and contractility [19]. Subsequently, a wide range of metabolic and neurological effects have been described (reviewed by Hoefig et al., Kohrle et al. and Zucchi et al. [20, 23, 33]). The former include decreased insulin and increased glucagon secretion, increased gluconeogenesis, and shift to lipid catabolism. Intracerebral T1AM administration caused a biphasic effect on feeding, sleep modulation with reduced non-REM sleep, increased locomotor activity, pro-learning and anti-amnestic effects, protection from toxic injury, increased autophagy, and reduced pain threshold. Recently, decreased cell proliferation was observed in several neoplastic cell lines exposed to T1AM [34, 35].

While TAAR1 might play a role in some of these effects, particularly in the neurological setting [36], other targets are likely to be involved, since several responses could be elicited also in TAAR1 knockout animals [23]. Determining which receptor is involved in which effect is a major target, and the underling signal transduction pathways are also poorly understood. Notably, modulation of gene expression significantly contributes to the metabolic effects [37–39].

As discussed for T2, to first step to evaluate the potential physiological role of T1AM is the comparison of endogenous levels with active dosages. The few available data show that neurological and metabolic effects are elicited with a relatively small increase (within one order of magnitude) over the baseline [37, 38, 40], while the gap is higher for cardiac effects or hypothermia. Further experiments and substantial technical advances will be needed to solve this important issue.

Investigations about the potential pathophysiological role of T1AM in humans are limited by the technical problems in the serum T1AM assay, which have been discussed above. So far, T1AM was reported to be increased in type II diabetes [26] and heart failure [30], while opposite changes occurred in NTIS [29]. Because of its effect on glucose homeostasis, T1AM increase in type II diabetes is particularly intriguing, although it is still unclear whether it plays a pathophysiological role, or is rather a consequence of the altered metabolic and endocrine state of these patients.

Interestingly, a positive correlation between serum T1AM and serum T3 or T4 was observed by Galli et al. [26] through a mass spectrometry-based technique. This may be consistent with the hypothesis that T1AM is produced in vivo from thyroid hormones, but it should be stressed that this finding was not confirmed in another investigation using an immunological assay [28].

T1AM has been proposed as a therapeutic agent, and promising preliminary results have been obtained in animal models of obesity [38, 41], dyslipidemia [38, 39], neuronal injury [42, 43], and myocardial ischemia–reperfusion [44]. Notably, endogenous thyronamines may not be the best candidates as novel therapeutic agents, because of their pleomorphic effects and complex pharmacokinetics. Therefore, several research lines are focused at developing synthetic thyronamine analogs and/or TAAR1 agonists [45], particularly for the treatment of obesity, but their evaluation is still at the initial stage.

Thyroacetic acids

It has been known since the 50's that TA4 (also known as Tetrac) and TA3 (also known as Triac) are produced in vivo

from T4 and T3, respectively [46, 47]. Their biosynthetic pathway requires T4/T3 decarboxylation and deamination. The original hypothesis [48] suggested that amine transferases or amine oxidases produce 3,5,3',5'-tetraiodothyropyruvic acid or 3,5,3'-triiodothyropyruvic acid, and then decarboxylation yields TA4 or TA3, respectively. While T4 and T3 are the substrates of thyroid hormone aminotransferase and of L-amino acid oxidase [1], decarboxylation has been classically attributed to aromatic amino acid decarboxylase (AADC), also known as L-DOPA-decarboxylase. This concept has been challenged, since purified AADC preparations could not decarboxylate iodothyronines [49]. The most recent hypothesis assumes that decarboxylation occurs first, yielding thyronamines (Fig. 3) [22], to be further oxidized by amine oxidases and aldehyde dehydrogenases, as discussed in the previous paragraph.

Deiodination may occur as the initial or final step in the pathway, since type I and II deiodinases can convert T4 into T3, or TA4 into TA3, but not T4AM into T3AM [50]. TA3 can be further deiodinated on both the phenolic (outer) and the tyrosyl (inner) ring, leading to the production of TA1 and thyroacetic acid (TA0), which is known to be excreted in human urine [51]. However, TA1 can also be produced from T1AM [20, 21], and in most tissues it appears to be the major T1AM catabolite (Fig. 2).

TA4 and TA3 have been assayed in human plasma by radioimmunological assays, at concentrations on the order of 40–700 pmol/l [52, 53] (Table 1). Parallel determinations by different techniques, particularly by LC–MS, would be valuable, since most antibodies raised against TA3 show up to 50% cross-reactivity with T3. TA3-free fraction is even lower than T3-free fraction, because its affinity for plasma proteins, particularly for transthyretin, is over tenfold higher [52]. In spite of tight protein binding, kinetic studies have shown that the clearance of thyroacetic acids is much quicker than observed for the corresponding thyronines, possibly due to their higher affinity for liver sulfotransferases and glucoronosyltransferases.

In addition to TA4 and TA3, TA1 has been detected in vivo, particularly in mouse brain homogenate, where its concentration was on the order of 300 pg/g (i.e., about 0.8 nmol/Kg) [54, 55]. Membrane transporters for thyroacetic acids appear to exist, and the cellular uptake of TA3 appears to be even quicker than observed for T3 [56]. Nonetheless, the specific transporters remain to be identified, as a role for the known T3/T4 transporters has been excluded [57].

TA3 is regarded as a potential thyromimetic, since it binds THRs with a similar of even higher affinity than T3: TA3 affinity for THR beta is about fivefold higher, while there is no significant difference with regard to THR alpha [58]. Another molecular target is the so-called plasma membrane integrin receptor, represented by integrin αvβ3, which mediates some noncanonical responses to thyroid hormones, probably thorough a MAP kinase-dependent pathway [11]. The latter appears to have particular importance in the stimulation of cell growth and of angiogenesis. At this site, T3 and T4 are virtually equipotent, while TA3 and TA4 are about fivefold less potent [52]. Notably, TA4 has an inhibitory action on the integrin-mediated effects of T3, although it is still unclear whether TA4 acts as a proper antagonist or rather as a reverse agonist [59].

Consistent with these molecular properties, the administration of exogenous TA3 (or TA4) at sufficiently high dosages produces widespread thyromimetic effects [52]. A specific therapeutic indication is represented by the so-called resistance to thyroid hormone syndrome, also known as Refetoff's syndrome [60]. This is caused by a set of mutations in THRs or proteins involved in the synthesis of deiodinases, which prevent adequate T3 binding and/or THR activation, and lead to a hypothyroid phenotype with elevated serum T3 and T4 [61, 62]. Since TA3 may bind to mutated THR, it is effective in many of these patients [60].

Another potential indication is represented by the Allan–Herndon–Dudley syndrome (AHDS). This is a rare congenital disease due to MCT8 mutations, impairing T3 and T4 transport, especially in the brain [63]. As a consequence, thyroid function tests show elevated serum T3, low or low–normal serum T4, and normal TSH. The phenotype is a mixture of signs of hypothyroidism (especially in the brain) and thyrotoxicosis (especially in the heart and liver). Since its cellular transport is not dependent on MCT8, TA3 is under investigation as a potential therapy for AHDS. A phase 1 clinical trial has been undertaken (five children aged 8 months to 6 years), and its results should be available shortly.

Because of its inhibitory effect on the integrin receptor, TA4 has been proposed as a therapeutic agent to slow neoplastic proliferation and neoangiogenesis [59, 64]. Interesting results have been reported with synthetic preparations, in which TA4 has been covalently linked to poly (lactic-co-glycolic acid) (PLGA) nanoparticles, in order to limit its distribution to the vascular compartment and to prevent systemic thyromimetic effects [65, 66]. The potential clinical exploitation of this strategy remains to be investigated.

The physiological or pathophysiological role of TA3 and TA4 is questionable, since endogenous TA3 and TA4 levels appear to be much than required to produce significant functional effects. However, a significant increase of plasmatic and urinary TA3 has been reported in fasting and in nonthyroidal illness syndrome [67]. The implications of these findings, if any, have not been determined so far.

TA1 has recently been added to the list of active thyroid hormone metabolites. While there is no evidence that TA1

can interact with THRs, TAAR1, or other known molecular targets, the administration of exogenous TA1 has produced significant functional effects. In mouse, intracerebroventricular injection of TA1 was associated with behavioral evidence of a pro-learning and anti-amnestic effect. In addition, the pain threshold was reduced and blood glucose increased [54]. More recently, it has been reported that exogenous TA1 administration has a protective effects versus kainic acid neurotoxicity and pentylenetrazol-induced seizures [42]. Notably, it has been proposed that some effects originally ascribed to T1AM may actually be mediated by TA1 [54, 55].

Conclusions

The field of thyroid hormone signaling has definitely grown more complex in recent years. The classical, or "canonical" view holds that T4 is a pro-hormone, and T3 is the active hormone, acting by modulating gene expression through THRs. It is now clear that some T3 effects do not require direct THR binding to DNA, or are even independent from THRs [68]. In addition, some thyroid hormone derivatives might act as independent chemical messengers. In general, a chemical messenger is an endogenous substance, able to interact with specific receptors and to produce functional effects at physiological concentration. We have discussed these issues with regard to several endogenous thyroid hormone metabolites. So far, no metabolite clearly fulfills all these requirements, but promising evidence has been collected for some of them, particularly T1AM.

The potential implications of this concept for human disease are unclear, but intriguing. Preliminary reports of changes in the blood levels of T1AM, T2, or TA3 in diabetes, heart failure, or non-thyroidal illness syndrome have been published, although their pathophysiological role, if any, is still unknown. On the other hand, the responses elicited in experimental animals have suggested potential therapeutic applications, and stimulated the development of synthetic analogs. TA3 has an established role in thyroid hormone resistance syndromes, and is under investigation in AHDS. Other potential targets are represented by obesity and dyslipidemia (for T2 and T1AM); dementia and degenerative brain disease (for T1AM and TA1); cancer (for T1AM and TA4).

The most important therapeutic indication for thyroid hormone is by far represented by replacement therapy in primary hypothyroidism or after total thyroidectomy. A major problem is that 10–15% of patients on replacement therapy with T4 complain of symptoms like asthenia, difficulty in concentration, and reduced cognitive performance, in spite of normal serum T3, T4, and TSH levels [69]. This has been attributed to the difficulty in normalizing tissue (particularly, brain) T3 concentration with systemic T4 administration, and there is controversy as to whether T4/T3 association may provide better results. An additional and unexplored question is the potential role of active thyroid hormone metabolites, e.g., whether systemic T4 can normalize tissue T2 or T1AM content.

In conclusion, the field of "novel thyroid hormones" includes interesting research lines and intriguing therapeutic opportunities. While several crucial questions are still open, important advances are hopefully anticipated for the near future.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement This article is a review and therefore it does not contain any studies with human participants or animals performed by any of the authors.

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