Thyronamines—Past, Present, and Future

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Thyronamines (TAMs) are a newly identified class of endogenous signaling compounds. Their structure is identical to that of thyroid hormone and deiodinated thyroid hormone derivatives, except that TAMs do not possess a carboxylate group.

Despite some initial publications dating back to the 1950s, TAMs did not develop into an independent area of research until 2004, when they were rediscovered as potential ligands to a class of G protein-coupled receptors called trace-amine associated receptors. Since this discovery, two representatives of TAMs, namely 3-iodothyronamine (3-T_1AM) and thyronamine (T_0AM), have been detected *in vivo*. Intraperitoneal or central injection of 3-T_1AM or T_0AM into mice, rats, or Djungarian hamsters caused various prompt effects, such as metabolic depression, hypothermia, negative chronotropy, negative inotropy, hyperglycemia, reduction of the respiratory quotient, ketonuria, and reduction of fat mass. Although their physiological function remains elusive, 3-T_1AM and T_0AM have already revealed promising therapeutic potential because they represent the only endogenous compounds inducing hypothermia as a prophylactic or acute treatment of stroke and might thus be expected to cause fewer side effects than synthetic compounds.

This review article summarizes the still somewhat scattered data on TAMs obtained both recently and more than 20 yr ago to yield a complete and updated picture of the current state of TAM research. **(*Endocrine Reviews* 32: 64–80, 2011)**

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Abbreviations: Adra$_{2A}$, α_{2A} Adrenergic receptor; DAT, dopamine transporter; Dio, deiodinase; DITPA, 3,5-diiodothyropropionic acid; GPCR, G protein-coupled receptor; icv, intracerebroventricular; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LP, long photoperiod; NET, norepinephrine transporter; PI3K, phosphoinositide 3-kinase; RQ, respiratory quotient; SP, short photoperiod; SULT, sulfotransferase; TA, thyroacetic acid; TAAR, trace-amine-associated receptor; TAM (or T_0AM), thyronamine; 3-T_1AM, 3-iodothyronamine; TH, thyronine; TR, T_3 receptor; UCP, uncoupling protein; VMAT2, vesicular monoamine transporter 2.

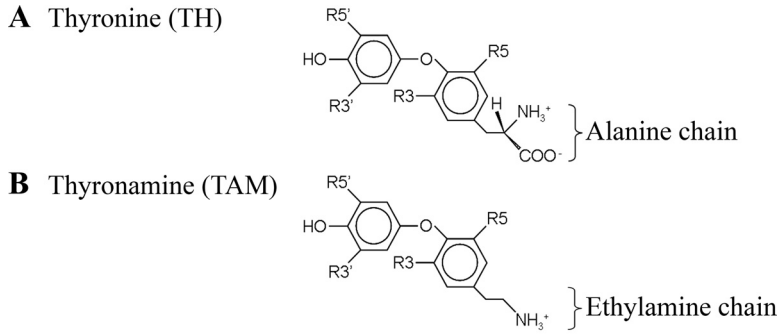
I. Introduction

The history of thyronamine (TAM) research appears peculiar. From 1951 to 1984, a few papers were published now and then, but most lacked any topical consistency. In 2004, after a 20-yr gap in TAM research, TAMs were rediscovered as potential ligands to a new class of G protein-coupled receptors (GPCRs) now referred to as trace-amine-associated receptors (TAARs) (1–3). Since this discovery, the field of TAM research has regained considerable momentum.

II. Structure

TAMs are a novel class of endogenous signaling compounds. In terms of structure, they differ from thyroid (pro-)hormone L-T_4 (T_4) and deiodinated thyronine (TH) derivatives only concerning the absence of the carboxylate group of the alanine side chain. THs and TAMs are designated T_x and T_xAM, respectively, with "x" indicating the number of iodine atoms per molecule, thus following the same rules for nomenclature. As illustrated in Fig. 1, there are nine THs and TAMs that differ by either the number or the position of the iodine atoms.

So far, only the three-dimensional structure of T_3AM has been published (4). In an attempt to compare the conformational properties of T_3AM with those of the corresponding thyronine T_3 and to examine the relative influence of the ethylamine side chain on the diphenylether conformation, the borosalicylate salt of T_3AM was subjected to crystallographic analysis. The experiments revealed that the overall molecular conformation of T_3AM was similar to that of T_3, displaying a twist-skewed conformation of the diphenylether portion and a proximal orientation of the 3′-iodine atom. These observations suggested that the amino group is of key importance in the determination of the conformation of T_3AM.



Analyte	R3	R5	R3'	R5'
T_0(AM)	H	H	H	H
3-T_1(AM)	I	H	H	H
3'-T_1(AM)	H	H	I	H
3,5-T_2(AM)	I	I	H	H
3,3'-T_2(AM)	I	H	I	H
3',5'-T_2(AM)	H	H	I	I
T_3(AM)	I	I	I	H
rT_3(AM)	I	H	I	I
T_4(AM)	I	I	I	I

FIG. 1. A, Structure of THs. B, Structure and nomenclature of TAMs. R, Variable residue.

III. *In Vivo* Detection

So far, only two representatives of TAMs, namely 3-iodothyronamine (3-T_1AM) and thyronamine (T_0AM), have been detected *in vivo* using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method (3, 5). Both compounds were detected in blood, heart, liver, adipose tissue, thyroid, and brain of adult male C57BL/6 mice, as well as in brain of several other species, such as Long-Evans rats and guinea pigs (3). 3-T_1AM was also detected in blood and brain of Djungarian hamsters (6).

The presence of the other TAMs *in vivo* has not been reported yet. In the LC-MS/MS studies by Scanlan *et al.* (3), the samples were simultaneously monitored for T_0AM, T_1AM, and T_2AM presence, but no T_2AM isomer was detected in any of the tissues. Likewise, T_3AM has not been detected from biological sources so far (7, 8). In early experiments applying butanolic extraction and subsequent paper chromatographic separation, ^{131}I-labeled T_4AM was found in plasma and in the thyroid glands, but not in the thyroid-derived thyroglobulin fractions of rats treated with ^{131}I (7). Furthermore, ^{131}I-labeled T_4AM, but not T_3AM, was detected in plasma of two patients who suffered from metastasizing thyroid tumors and were treated with high doses of ^{131}I. Based on these observations, T_4AM was postulated to be present *in vivo* at a serum concentration equaling 1 to 2% of the serum T_4 concentration (7, 9). However, T_4AM biosynthesized from

endogenous nonradioactive iodine precursors has not been described yet.

Hitherto, several publications have reported on endogenous TAM concentrations in tissues. 3-T_1AM was estimated to be present in brain of Long-Evans rats at subpicomole per gram quantities (3). This concentration is lower compared with 1 to 6 pmol/g T_4 reported for rat brain using a RIA technique (10). Although this concentration seems low at first glance, it was emphasized that the cell type-specific and subcellular distributions of TAMs are unknown. Therefore, their effective concentrations within a given cell type and in specific cellular compartments might be significantly higher than the average whole tissue concentrations measured. In the hearts of male Wistar rats, the endogenous 3-T_1AM concentration averaged 68 pmol/g wet weight and showed a wide range of variation from 1 to 120 pmol/g wet weight (11). Interestingly, the average value of 68 pmol/g wet weight corresponds to the cardiac content of classical modulators of heart function, such as adrenaline, dopamine, and adenosine (11). Moreover, it exceeds the endogenous intracardial T_3 and T_4 concentrations by a factor of 20 and 2, respectively (11). Even if it was not stated whether the hearts had been perfused, these findings indicate that TAMs can override TH concentrations locally in the heart. In serum of Djungarian hamsters, endogenous 3-T_1AM was detected at a concentration of roughly 6 nM (6).

In 2008, Geraci *et al.* (12) showed at the American Thyroid Association (ATA) meeting in Chicago, Illinois, the first endogenous 3-T_1AM concentration in human tissues (higher concentrations in thyroid, skeletal muscle, adipose tissue, and prostate, ~60 nM; n = 2–5) and serum (~60 nM; n = 16) using a solid phase extraction and LC-MS/MS method described by DeBarber *et al.* (5). Independently of the Scanlan group (12), Soldin *et al.* (13) described at the 2009 ATA meeting in Palm Beach, Florida, a novel analytical method applying LC-MS/MS for the simultaneous identification and quantification of THs and TAMs in biological samples using today's most sensitive triple quadrupole mass spectrometer (API 5000 LC/MS/MS System). Using an existing procedure for preanalytical extraction of THs and TAMs from mouse tissues and a previously validated LC-MS/MS analytical method published by Piehl *et al.* (14), mouse tissue 3-T_1AM levels in the range of 1 to 20 pmol/g were determined (F. Wohlgemuth, C. S. Hoefig, and J. Köhrle, unpublished data).

But there are still controversial discussions in the scientific community about the detection and quantification of endogenous 3-T_1AM. Part of this controversy might be related to the strong binding of 3-T_1AM to apolipoprotein B100 in human serum, which leads to very low free TAM concentrations in the range around 60 nM or lower (Ref. 12, and our unpublished data). Surprisingly, 3-T_1AM and T_0AM appear to prefer different serum proteins for avid binding compared with THs, which strongly bind to T_4 binding globulin, transthyretin, albumin, and to some extent also to lipoproteins (15). Currently, no validated and peer-reviewed procedure has been published that allows for the concomitant and simultaneous determination of both the TH and the TAM profiles from one whole blood, serum, or plasma sample using an efficient and reproducible extraction procedure and LC-MS/MS analytics.

IV. Systemic and Local Effects

The physiological roles of TAMs remain elusive. The first documented biological activity of TAMs was reported for their remarkable sensitization of the inhibitory effect of 10^{-6}/cm^3 adrenaline on the isolated intestine of thyroidectomized rabbits. Surprisingly, triiodothyronamine (probably 3,3′,5-triiodothyronamine) was the most potent compound (10^{-18}) compared with thyroxamine (10^{-14}) and diiodothyronamine (10^{-5}), but unfortunately it has not been clearly specified whether rT_3AM or T_3AM was used and which of the three T2-thyronamine isomers was actually tested (16). Compared with the thyronines T_4 (10^{-11}) and triiodothyronine (10^{-15}), the TAMs were more potent and acted immediately without the latency period observed for the THs (16). These authors already proposed an *in situ* decarboxylation of THs to TAMs by hypothyroid rabbit intestinal tissue. In further studies from the 1950s, T_4AM and T_3AM were only 10% as potent as T_4 in terms of goiter prevention and stimulation of oxygen consumption in rats (17, 18). However, T_3AM was 36 times as potent as T_4 at inducing tadpole metamorphosis (18). In 1974, it was suggested that TAMs might be derived from THs in adrenergic neurons to act as "thyronergic" neurotransmitters (19). This suggestion was based on preliminary evidence that some enzymes involved in catecholamine biosynthesis, including aromatic L-amino acid decarboxylase, might use T_4 as an alternate substrate.

In more recent studies, ip injection of 3-T_1AM or T_0AM into C57BL/6 wild-type mice or Djungarian hamsters caused various prompt effects, such as metabolic depression, hypothermia, negative chronotropy, negative inotropy, hyperglycemia, reduction of the respiratory quotient (RQ), ketonuria, and reduction of fat mass (3, 6). In most of these studies, a dose of 50 mg 3-T_1AM or T_0AM/kg body weight was injected (~140 μmol 3-T_1AM/kg body weight and ~218 μmol T_0AM/kg body weight). In Djungarian hamsters, this dose leads to an approximately 10-fold increase in 3-T_1AM blood concentrations from approximately 6 nM to roughly 56 nM (6).

TABLE 1. Summary of the pharmacological effects of TAMs identified so far

	Experimental model	Mechanism and/or interpretation	Refs.
Central nervous effects			
Hypothermia	C57BL/6 mice, Djungarian hamsters	Due to a decrease in metabolic rate	3, 6, 20
Inhibition of monoamine transporters	Transfected cell lines	Hypothesized action as a physiologically relevant neuromodulator	31
Cardiac effects			
Negative chronotropy,[a] negative inotropy	C57BL/6 mice, isolated rat hearts	Due to effects on intracellular calcium homeostasis	3, 11, 22–24
Effects on plasma hormone concentrations			
Hyperglycemia, hypoinsulinemia, hyperglucagonemia	Primary human and murine pancreatic β-cells, male Wistar rats, ROSA26PTX mice	Hypothesized central action increasing the sympathetic tone on pancreatic islets	27, 28
Hypothalamic-pituitary-thyroid-axis: TSH ↓, T_4 ↓, T_3 ↓	Male Wistar rats	Causing a state reminiscent of the nonthyroidal illness	28
Metabolic effects			
Metabolic rate ↓, RQ ↓, lipid utilization ↑, fat mass ↓, ketonuria	C57BL/6 mice, Djungarian hamsters	Hypothesized action as a physiologically relevant modulator of metabolism: change in metabolic fuel utilization from carbohydrates to mainly lipids	6

↓, Reduction; ↑, increase.

[a] Conflicting data have been reported for the cardiac effects of T_0AM (11, 25, 26).

Currently, it is not known whether such changes in TAM blood and tissue concentrations have to be considered as physiological or pharmacological. Therefore, the effects of TAMs described so far will conditionally be classified and described as pharmacological effects herein (Table 1).

The majority of the pharmacological effects of 3-T_1AM described so far were detected within minutes after injection and displayed roughly similar time courses. However, the respective amplitudes of metabolic depression, negative chronotropy and inotropy, hypothermia, hyperglycemia, and RQ reduction were reached at different time points, namely at 5 min, less than 10 min, 30 min, 2.5 h, and 3 to 4.5 h after injection, respectively (3, 6, 11). Furthermore, whereas the metabolic rate, heart activity, body temperature, and blood glucose levels were normalized 6 to 8 h after injection, the reduced RQ was maintained for more than 24 h (6). However, ketonuria and the reduction of fat mass were detected and reversed much later, compared with the aforementioned acute effects of 3-T_1AM injection.

A. Reduction of metabolic rate by 3-T_1AM

Intraperitoneal injection of 3-T_1AM into C57BL/6 wild-type mice and Djungarian hamsters caused a reduction of metabolic rate measured as a decrease in VO_2 from 2.5 to 1.8 ml/g · h within 5 min (6).

B. Hypothermia

Upon ip injection into C57BL/6 wild-type mice, 3-T_1AM and T_0AM reduced the body temperature within 30 min by roughly 8 C at EC_{50} of 59 and 178 μmol/kg body weight, respectively (Fig. 2) (3, 20, 21). The mice became inactive but reflexes were retained, indicating that they were not anesthetized. Furthermore, their skin got cool to the touch, but they did not display any compen-

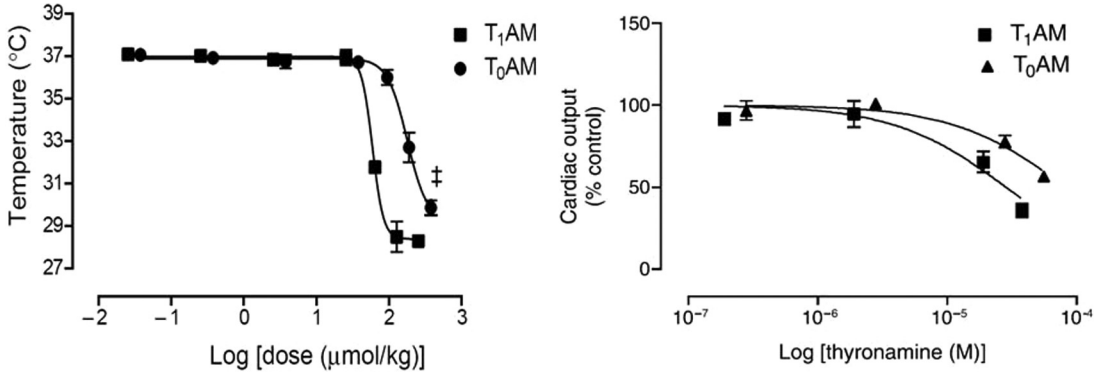


FIG. 2. Pharmacological effects of 3-T_1AM and T_0AM on body temperature (*left*) and cardiac output (*right*) in mice. ‡, indicates significant difference between two treatment groups. [Modified with permission from T. S. Scanlan *et al.*: *Nat Med* 10:638–642, 2004 (3). Copyright Nature Medicine.]

satory reactions, such as shivering, huddling, or piloerection. The effect was completely reversible after 6 to 8 h and, so far, no side effects were reported even after repeated treatments over a period of 2 months (3).

Although less pronounced, this hypothermic effect was also observed after ip injection of 50 mg (~140 μmol) 3-T_1AM/kg body weight into Djungarian hamsters (6).

In both wild-type C57BL/6 mice and Djungarian hamsters, the amplitude of hypothermia caused by 3-T_1AM injection (30 min) was preceded by the amplitude of the reduction in metabolic rate (5 min) (6). Because the extent in hypothermia and the amount of metabolic reduction were highly correlated, the hypothermic effect was interpreted as a result of the reduced metabolic rate (6).

C. Negative chronotropy and negative inotropy of 3-T_1AM

The hypothermic and metabolic effects of 3-T_1AM ip injected into wild-type C57BL/6 mice were accompanied by reversible bradycardia (3). The amplitude of the effect was measured 1 h after injection when the heart rate had dropped from approximately 600 to approximately 350 beats per minute. Bradycardia reversed to normal 6–8 h after injection. This negative chronotropic effect of 3-T_1AM was found to be direct by perfusing an *ex vivo* adult rat denervated working heart preparation with increasing 3-T_1AM concentrations. 3-T_1AM reduced the heart rate within minutes at a half maximal IC_{50} of 38 μM. The effect persisted throughout the experiment (60 min) and was reversed by removing 3-T_1AM from the perfusion medium (11). In addition, 3-T_1AM was even more potent (IC_{50} = 29 μM) at decreasing the cardiac output of the perfused rat hearts (Fig. 2). The amplitude of this negative inotropic effect was measured 10 min after application. Consistent with the negative chronotropic effect observed, the negative inotropy also persisted throughout the experiment (1 h) (3) and was reversible by removing 3-T_1AM (11). Taken together, 3-T_1AM elicits chronotropic and inotropic effects on the heart with different potencies (3), which is remarkable considering that only a few endogenous negative inotropic agents such as adenosine, TNFα, and IL-6 have been identified so far (11). Using the *ex vivo* adult rat denervated working heart preparation, 3-T_1AM also inhibited all further cardiac parameters measured, including systolic aortic pressure (3) and coronary flow (11).

Studies employing isolated perfused rat hearts revealed that 20 μM 3-T_1AM decreased cardiac contractility but did not alter glucose uptake or oxygen consumption compared with mechanical work (22). Only at 25 μM 3-T_1AM, oxygen consumption decreased. Patch clamp analyses on adult rat cardiomyocytes demonstrated altered calcium and potassium homeostasis, including a diminished calcium pool in the sarcoplasmic reticulum (22). Twenty micromoles of 3-T_1AM increased the diastolic leak through the ryanodine receptor leading to the negative inotropic action. Furthermore, a longer duration of the action potential was observed, which might be related to 3-T_1AM inhibiting transient outward I_{to} and I_{K1} background currents. 3-T_1AM decreased both the amplitude and the duration of depolarization-induced calcium transients at these pharmacological concentrations. However, no evidence for 3-T_1AM effects on protein kinase A activity was observed in these studies (22).

The negative chronotropic and inotropic effects of exogenous 3-T_1AM as well as the increased resistance to ischemic injury, possibly due to its actions on intracellular calcium homeostasis, have been reviewed comprehensively elsewhere (23, 24).

D. Negative chronotropy of T_0AM

In the perfused, denervated rat heart, T_0AM decreased the cardiac output within minutes at an IC_{50} of 83 μM, whereas no effect on heart rate was observed (Fig. 2) (3). Because the effect was not abolished by pacing the hearts, it was concluded that T_0AM is a negative inotropic agent but (in contrast to 3-T_1AM) not a negative chronotropic agent (11).

Those results are inconsistent with a number of earlier studies using different models. T_0AM (9.4 to 94 μM) displayed positive inotropic effects and did not modify the heart rate in an isolated guinea pig atria model (25). In anesthetized rats, 3 to 30 mg T_0AM/kg body weight (13 to 131 μmol T_0AM/kg body weight) decreased the heart rate significantly (25). Upon iv injection into anesthetized dogs, T_0AM increased the cardiac output (25, 26). However, T_0AM induced concomitant negative chronotropy in one study (25) but positive chronotropy in the other study (26).

In summary, conflicting data have been reported for the cardiac effects of T_0AM.

E. Hyperglycemia

In addition to hypothermia and metabolic suppression, C57BL/6 wild-type mice ip injected with 50 mg (~140 μmol) 3-T_1AM/kg body weight displayed an increase in blood glucose level (27). This hyperglycemic effect was detectable within minutes after treatment, reaching a maximum of 250% above basal 2 h after 3-T_1AM injection and was completely reversed 8 h after injection. It was accompanied by a decrease in plasma insulin levels and an increase in plasma glucagon levels. Insulin applied exogenously 2 h after 3-T_1AM injection normalized the blood glucose levels, indicating that the peripheral tissues had remained sensitive to insulin during the 3-T_1AM treatment (27). Consistent with these findings, 3-T_1AM inhibited the glucose-stimulated insulin release from primary murine and human pancreatic β-cells *in vitro* (27).

In a more recent study, ip injection of 50 mg (~218 μmol) T_0AM/kg body weight into rats was also reported to increase the plasma concentrations of glucose and glucagon, albeit to a lesser extent than 3-T_1AM (28).

F. Reduction of the respiratory quotient

Also within minutes after ip injection of 3-T_1AM, the RQ decreased from 0.9 to 0.7 in both C57BL/6 wild-type mice and Djungarian hamsters (6). This was indicative of a rapid change in metabolic fuel utilization from predominantly carbohydrates (RQ, ~0.9) before treatment to primarily lipids (RQ, ~0.7) after treatment (6). Minimal RQ values were obtained 3 to 4.5 h after treatment. The reduced RQ was maintained for more than 24 h, *i.e.*, even after recovery of the metabolic rate, and it only returned to normothermia and normalization of cardiac function much later. This indicated that treated animals required several hours to readjust their metabolic machinery to normal catabolism (6).

G. Ketonuria and reduction of fat mass

The assumption that energy requirements were primarily covered by lipid utilization in response to 3-T_1AM treatment was confirmed by two distinct observations. First, ketone bodies were detected in the urine of Djungarian hamsters from 8 to 28 h after injection (6). Second, a significant loss of body weight due to a loss of fat mass was measured from d 1 to 4 and 6 in summer-acclimatized [long photoperiod (LP)] and winter-acclimatized [short photoperiod (SP)] hamsters, respectively (6).

H. 3-T_1AM does not induce torpor

The initial reports on profound hypothermia, bradycardia, and reduced cardiac output caused by injection of 3-T_1AM into C57BL/6 wild-type mice prompted several researchers to question whether 3-T_1AM acted as a hormonal cue for torpor initiation. To address this question, the effects of 3-T_1AM injection were compared between C57BL/6 wild-type mice, SP and LP Djungarian hamsters. Although mice and LP hamsters do not display spontaneous torpor (29, 30), SP hamsters are seasonally prepared to enter torpor spontaneously, even if fed *ad libitum* (6). However, the metabolic depression via 3-T_1AM was greater in mice than in hamsters and also more pronounced in LP hamsters compared with SP hamsters. Furthermore, the amplitudes of metabolic reduction and hypothermia triggered by 3-T_1AM were less pronounced compared with torpor. Because additionally the time course of the decrease in metabolic rate and RQ mediated by 3-T_1AM were inconsistent with those observed in torpor, 3-T_1AM was excluded as the major hormonal stimulus for spontaneous daily torpor (6).

I. Application routes other than ip and doses other than 50 mg/kg body weight

A recent study demonstrated that compared with the regular ip injection of 50 mg 3-T_1AM or T_0AM/kg body weight, intracerebroventricular (icv) infusion of a 100-fold lower dose of 3-T_1AM or T_0AM (0.5 mg/kg body weight) produced even more pronounced effects on peripheral glucose metabolism in male Wistar rats (28). No leakage of the icv applied substances into peripheral compartments was found. These observations support a central action of 3-T_1AM and T_0AM on hepatic glucose output and metabolism. The authors suggested an increased sympathetic tone on pancreatic islets via central TAM effects as the underlying mechanism. In addition, sympathetic and parasympathetic projections might activate hepatic glucose production. Furthermore, a direct TAM effect, stronger for 3-T_1AM than for T_0AM, on the activation of the hypothalamic-pituitary-adrenal axis has to be postulated to account for the increase in corticosterone serum concentration induced by 3-T_1AM and T_0AM, which was also observed in these studies. Although systemic administration of T_1AM and T_0AM decreased plasma TSH, T_4, and T_3, no such changes were found after icv injection of the low TAM doses.

A recent independent study demonstrated that direct 3-T_1AM injection into the arcuate nucleus caused a 3-fold increase in food intake in rodents, suggesting that the orexigenic effect of 3-T_1AM is not caused by energy expenditure but rather is regulated on the hypothalamic level (21). Intraperitoneal or icv injection of low doses of 3-T_1AM (4 and 1.2 μmol/kg body weight, respectively) into rats or mice caused a significant increase in food intake without affecting oxygen consumption and locomotor activity. However, at high 3-T_1AM doses (50 mg/kg body weight, ~127 μmol), the authors confirmed the previously reported reduction of oxygen consumption and locomotor activity (3). Stimulation of primary hypothalamic explants with 3-T_1AM led to a significant increase of neuropeptide Y release, which might account for the increased food intake observed in the *in vivo* experiments.

J. Inhibition of monoamine transporters

In an attempt to elucidate the mechanisms associated with the *in vivo* effects of TAM, the interactions between TAMs and neuronal monoamine transporters were studied. These investigations were motivated by the presence of TAMs in brain and by their structural similarity to monoamine transmitters and trace amines (31). In heterologous expression systems, all TAMs except rT_3AM inhibited the activity of the plasma membrane human and rat dopamine transporter (DAT). Similarly, all TAMs except T_4AM inhibited the rat vesicular monoamine transporter 2 (VMAT2), which is not localized to the plasma

membrane but to the intracellular vesicular membrane. By contrast, the plasma membrane-bound human and rat serotonin transporter was inhibited exclusively by 3,3′-T_2AM, but it was not affected by the remaining TAMs. Furthermore, the plasma membrane-bound human norepinephrine transporter (NET) exhibited a mixed behavior becoming activated by several TAMs and inhibited by others, including 3-T_1AM. In these heterologous expression systems, 3-T_1AM inhibited DAT, VMAT2, and NET at IC_{50} values in the lower micromolar range and displaying either a competitive or a mixed mode of inhibition. In both the heterologous expression system and primary rat brain synaptosome or synaptic vesicle preparations, 3-T_1AM was confirmed as an inhibitor and excluded as a substrate of DAT, VMAT2, and NET. Therefore, 3-T_1AM is the only known endogenous phenethylamine identified to date that inhibits VMAT2 but fails to be recognized as a substrate. Taken together, the potencies of TAMs to inhibit monoamine transporters were comparable to the potencies of the biogenic trace amines, which also display IC_{50} values in the lower micromolar range (31). Accordingly, the inhibition of DAT and NET by TAMs, which might lead to extracellular monoamine accumulation, and the inhibition of VMAT-2 by TAMs, which is likely to cause a depletion of neurotransmitter stores and subsequent reduction of further monoamine signaling, might have a physiological relevance (31).

V. Receptors

To this end, the physiological receptor(s) of TAMs has not been identified unambiguously. Although some receptors have been excluded as candidates, some experimental evidence has been gathered for others to qualify as potential receptors mediating TAM signaling (1).

A. Early descriptions of thyronamine binding sites

T_4AM displaced ^{125}I-labeled T_3 or T_4 from binding to cytosolic and nuclear protein fractions obtained from human leukocytes at apparent K_i values in the lower nanomolar range, *i.e.*, comparable to unlabeled T_3 and T_4, respectively (32, 33). T_3AM, 3,5-T_2AM, and T_0AM did not only interfere with ligand binding to β-adrenergic receptors expressed at the plasma membrane of turkey erythrocytes but also inhibited the downstream activation of cAMP synthesis (4, 8). Notably, these TAMs exhibited apparent K_i values comparable to the K_i of isoproterenol, which is a well-established β-adrenergic receptor ligand. By contrast T_4, T_3, rT_3, and 3,3-T_2 failed to show any effect (4, 8). From these studies, it was concluded that T_3AM, 3,5-T_2AM, and T_0AM bind to β-adrenergic receptors but do not activate cAMP signaling. Instead, they prevented cAMP signaling by isoproterenol. However, in a more recent study, the application of 1 μM isoproterenol to isolated, denervated *ex vivo* rat hearts pretreated with 38 μM 3-T_1AM caused a reversal of heart rate, cardiac output, and systolic aortic pressure to baseline, within 30 sec to 1 min. Accordingly, it was concluded that signaling via β_1 adrenergic receptors is preserved in the presence of 3-T_1AM (3). The reasons for the discrepancy between these studies remain elusive.

B. Receptors for thyroid hormones and biogenic amines

The structural similarities of TAMs with THs and biogenic amines suggested that TAMs might signal via nuclear thyroid hormone receptors or receptors of biogenic amines. On the other hand, the rapid onset of TAM effects had not only led to their early classification as "nongenomic effects" but also motivated the proposal that TAMs might not signal via slowly acting nuclear thyroid hormone receptors (34).

In an early study, T_3AM did not displace ^{125}I-T_3 from isolated rat liver nuclei (4). In line with this, T_0AM and 3-T_1AM were reported to neither bind to nor activate nuclear thyroid hormone receptors using nuclear receptor binding and reporter gene assays (3). Furthermore, neither T_0AM nor 3-T_1AM activated the heterologously expressed $G_{\alpha s}$-coupled dopamine D_1 and β_2 adrenergic receptor (3).

Thus, despite their structural similarities, TAMs, THs, and certain biogenic amines appear to signal via different receptors.

C. Trace amine-associated receptor 1

The first receptor suggested to mediate TAM effects was the membrane-bound G protein-coupled TAAR-1 (3). This suggestion was based on the finding that 3-T_1AM, 3,3′-T_2AM, 3,5-T_2AM, T_3AM, and T_0AM stimulated the intracellular accumulation of cAMP in HEK-293 cells stably transfected with rat TAAR-1, with EC_{50} values ranging from 14 to 131 nM with respect to the indicated order (3). Analysis of structure-activity relationships of single and double mutant rat and mouse TAAR-1 stably expressed in HEK-293 cells revealed surprisingly distinct, species-specific ligand preferences for a series of TAMs in comparison to 3-T_1AM. Residues critical for the distinct, high-affinity ligand binding were identified in the transmembrane helices 4 and 7 of TAAR-1 of both species (35). Given the structural similarity between TAMs and biogenic amines, the expression of TAAR-1 transcript in TAM target tissues such as heart (11), brain (2), and pancreas (27), as well as the specific binding of [3-^{125}I]T_1AM to cardiac membrane preparations, TAAR-1 has been considered an excellent candidate receptor for T_0AM and

3-T_1AM (20). This notion was further supported by the observation that synthetic analogs of 3-T_1AM exhibiting comparable or even higher potencies than 3-T_1AM at activating heterologously expressed TAAR-1 also induced hypothermia after ip injection into mice (36). These synthetic derivatives included a 3-T_1AM-like compound lacking the 4′-OH group, an N-methylated 3-T_1AM derivative containing a fluorine atom instead of the 4′-OH group, and a derivative with a methyl group at the 3-position of the TAM scaffold.

However, the concept of TAM signaling via a $G_{\alpha s}$PCR, such as TAAR-1, has been challenged. In parallel experiments of the study by Scanlan *et al.* (3) using murine TAAR-1, only 3-T_1AM and 3,5-T_2AM activated cAMP accumulation at EC_{50} values in the nanomolar range, whereas all other TAMs were unable to trigger cAMP synthesis at concentrations of 1 μM or less. Likewise, perfusion of isolated rat hearts with 3-T_1AM did not increase intracardial cAMP level, although transcripts of several TAAR isoforms including TAAR-1 were detected in rat heart and specific binding of [3-^{125}I]-T_1AM to cardiac membrane preparations was observed (11). Moreover, the β-adrenergic receptor agonist isoproterenol, which increases the intracardial cAMP concentration upon activation of the cardiac β_1 adrenergic receptor, reversed the negative chronotropic and inotropic effects of 3-T_1AM in the perfused rat heart (3). In line with this, T_3AM, 3,5-T_2AM, and T_0AM inhibited the cAMP synthesis in turkey erythrocytes, which had been prestimulated with isoproterenol (4). Moreover, 3-T_1AM did not have any effect on blood glucose and insulin levels in a mouse model carrying a $G_{\alpha i}$PCR knockout specific to pancreatic β-cells (27). *Bordetella pertussis* toxin, which ADP-ribosylates and uncouples all $G_{\alpha i}$ proteins except G_z from their upstream GPCRs, prevented the negative effect of 3-T_1AM on the glucose-stimulated insulin release from primary murine and human pancreatic β-cells (27).

In conclusion, the hypothermic, negative chronotropic, negative inotropic, and hyperglycemic effects of 3-T_1AM were more consistent with an inhibition of cAMP accumulation through $G_{\alpha i}$ activation than with an effect on $G_{\alpha s}$. Yet, TAAR-1 has not been withdrawn as a candidate TAM receptor for several reasons. The observed pharmacological TAM effects may be secondary to the direct activation of TAAR-1 (3). Furthermore, although TAAR-1 couples to $G_{\alpha s}$ in stably transfected HEK293 cells, it might couple differently in different tissues *in vivo* (3). However, in a *Xenopus* oocyte expression system in which the M2 muscarinic receptor readily coupled to $G_{\alpha i}$, no coupling of TAAR-1 to $G_{\alpha i}$ was detected (27).

However, a recent report rejects the hypothesis that TAAR-1 mediates the thermoregulatory response to 3-T_1AM and other trace amines (37). TAAR-1 knockout and wild-type male mice were used to prepare synaptosomes employed for binding and uptake assays, behavioral, and pharmacological studies with 3-T_1AM in comparison to other trace amines and amphetamine-like psychostimulants. Experiments using cells transfected with rhesus monkey TAAR-1 and human DAT complemented these studies. Although 3-T_1AM competed for DAT uptake as well as for serotonin uptake in these models, 3-T_1AM-induced TAAR-1 signaling was not increased in DAT-transfected cells. Surprisingly, the previously demonstrated impressive hypothermic cryogenic response to 3-T_1AM administration was maintained in TAAR-1 knockout mice to a similar extent and with the same dose response at 25 and 50 mg/kg (~70 and 140 μmol 3-T_1AM/kg) body weight. Whether other members of the TAAR family or other plasma membrane receptors mediate this impressive cryogenic 3-T_1AM response, therapeutically relevant for treatment of stroke, cardiovascular complications, and intensive care medicine, remains to be studied.

D. Alpha$_{2A}$ adrenergic receptor

A second receptor proposed to mediate the effects of 3-T_1AMs was the α_{2A} adrenergic receptor (Adra$_{2A}$), which is an established $G_{\alpha i}$ coupled receptor expressed in many cell types including pancreatic β-cells (27, 38). *In vitro* studies demonstrated a higher affinity of human and murine Adra$_{2A}$ for 3-T_1AM compared with epinephrine, an accepted endogenous ligand of Adra$_{2A}$ (27). Coadministration of the Adra$_{2A}$ antagonist yohimbine with 3-T_1AM to wild-type mice inhibited the hyperglycemic effects of 3-T_1AM. Moreover, 3-T_1AM had no effect on blood glucose and insulin levels in mice carrying a $G_{\alpha i}$PCR knockout specific to pancreatic β-cells and failed to induce hyperglycemia in Adra$_{2A}$-null mice (27).

E. Concerted function of TAAR-1 and Adra$_{2A}$?

So far, several lines of evidence indicate that Adra$_{2A}$ and TAAR-1 might play a concerted role in mediating the effects of 3-T_1AM on carbohydrate metabolism. As described above, 3-T_1AM inhibited the glucose-stimulated insulin release from primary murine and human pancreatic β-cells in a *Bordetella pertussis* toxin-sensitive fashion (27). However, 3-T_1AM stimulated insulin secretion from the MIN6 insulinoma cell line, which expresses TAAR-1 and Adra2 in a reversed ratio compared with murine islets. This effect was potentiated by *Bordetella pertussis* toxin or the Adra2A antagonist yohimbine. Moreover, 3-T_1AM did not only fail to induce hyperglycemia in Adra2A-null mice, but rather induced hypoglycemia (27). Taken together, 3-T_1AM might activate insulin secretion in pan-

creatic β-cells via $G_{\alpha s}$-coupled TAAR-1 and inhibit it via $G_{\alpha i}$-coupled Adra2a. Consistent with this model, the Adra2 effect dominates in primary pancreatic β-cells, which express higher concentrations of Adra2A than TAAR-1. Likewise, the TAAR-1 effect dominates in MIN6 cells in which the TAAR-1 expression is higher relative to that of Adra2.

VI. Signal Transduction Pathways Activated by Thyronamines

To assess the signaling pathways activated by TAMs more systematically, *ex vivo* adult rat working heart preparations, which had been pretreated with various inhibitors of signaling cascades, were perfused with T_0AM and 3-T_1AM (11). Remarkably, *Bordetella pertussis* toxin, the aforementioned inhibitor of $G_{\alpha i}$ signaling, did not interfere with the negative inotropic effects of T_0AM and 3-T_1AM, thus excluding a role of $G_{\alpha i}$ proteins for TAM effects on the heart. Likewise, inhibitors of various kinases, directed against protein kinase A, protein kinase C, Ca^{2+}-calmodulin kinase 2, phosphoinositide 3-kinase (PI3K), and MAPK2 did not show any effect. By contrast, the negative inotropic effects of 3-T_1AM were potentiated by genistein, a tyrosine kinase inhibitor, and attenuated by vanadate, a phosphotyrosine phosphatase inhibitor (11). Because vanadate also acts as a P-type ATPase inhibitor and thus possesses intrinsic inotropic effects, it could not be concluded directly that 3-T_1AM might signal by activating phosphotyrosine phosphatases. However, the detection of tyrosine phosphorylations and dephosphorylations in the cytosolic and microsomal fraction of rat ventricles perfused with 3-T_1AM strengthened the hypothesis that 3-T_1AM might signal via tyrosine phosphorylation and dephosphorylation pathways (11). In the same study, Ca^{2+} depletion or inhibition of phospholipase C also potentiated the negative inotropic effects of 3-T_1AM, indicating a more complex signaling by TAMs (11).

VII. Biosynthesis and Deiodination Reactions

The pharmacological effects reported for TAMs identified an interesting discrepancy between TAMs and classical THs. On the one hand, TAMs and THs are highly similar in physicochemical characteristics and structure (Fig. 1), but on the other hand the short-term hypothermic, negative chronotropic and negative inotropic effects of TAMs are opposite in direction to the actions of the classical active thyroid hormone T_3 (1). Thus, TAMs were suggested to be derivatives of thyroid hormones that might serve to fine-tune or even antagonize thyroid hormone effects (39).

Yet, the pathways of TAM biosynthesis are still unknown. If TAMs were derivatives of TH (*e.g.*, T_4 or T_3), decarboxylation of the alanine side chain would be required for their biosynthesis (1). So far, no TH decarboxylating enzyme has been identified, although some preliminary evidence that T_4 might be a substrate of aromatic L-amino acid decarboxylase has been reported since 1974 (4, 19).

This hypothesis could not yet be supported using human recombinant amino acid decarboxylase preparations and all THs as substrates, whereas its substrate L-DOPA (L-Dihydroxyphenylalanine) was readily decarboxylated to yield dopamine under comparable reaction conditions (Ref. 40 and our unpublished data).

If the putative decarboxylating enzyme was converting only THs with higher iodine content, deiodinases (Dio1, Dio2, and Dio3) would be directly required to complete 3-T_1AM and T_0AM biosynthesis by removing at least one to four iodine atoms. Intracellular Dios catalyze the reductive removal of iodine from their substrates (41). Because a role of Dios in TAM biosynthesis requires their ability to accept TAMs as substrates, it was investigated whether TAMs are converted by Dios (14, 42). TAMs were incubated with isozyme-specific Dio preparations. Deiodination products were analyzed using a newly established method applying LC-MS/MS. Phenolic ring deiodinations of rT_3AM, 3′,5′-T_2AM, 3,3′-T_2AM, as well as tyrosyl ring deiodinations of T_3AM and 3,5-T_2AM were observed with Dio1. These reactions were completely inhibited by the Dio1-specific inhibitor 6n-propyl-2-thiouracil. Dio2-containing preparations also deiodinated rT_3AM and 3′,5′-T_2AM at the phenolic rings, but in a 6n-propyl-2-thiouracil-insensitive fashion. All TAMs with tyrosyl ring iodine atoms were deiodinated at the tyrosyl ring by Dio3-containing preparations. In functional competition assays, the newly identified TAM substrates inhibited an established TH deiodination reaction. By contrast, TAMs, which had been excluded as Dio substrates in LC-MS/MS experiments, failed to show any effect in the competition assays, thus excluding them as substrates and verifying the former results.

These data support a role for Dios in TAM biosynthesis and contribute to confining the biosynthetic pathways for 3-T_1AM and T_0AM (Fig. 3) (14).

VIII. Metabolism

A. First findings

The first study analyzing potential metabolizing reactions of TAMs (43) was motivated by previous experiments demonstrating that ip injection of a synthetic 3-T_1AM derivative lacking the 4′-OH group into

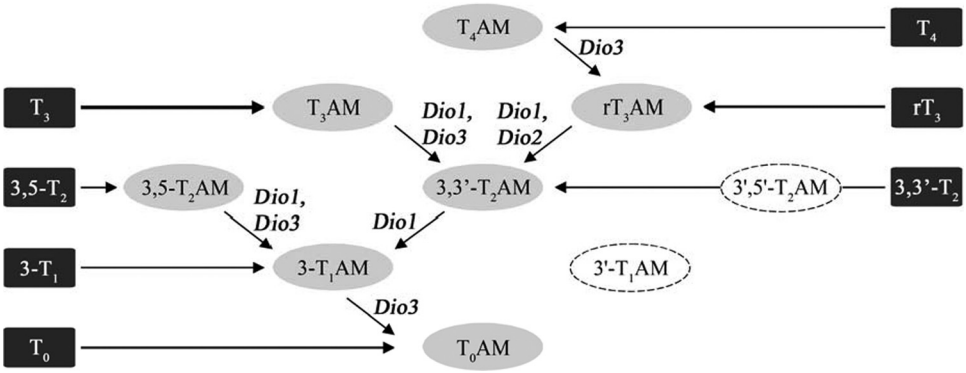


FIG. 3. Pathways suggested for TAM biosynthesis. *Diagonal arrows*, Phenolic ring deiodination reactions catalyzed by Dio1 or Dio2 and tyrosyl ring deiodination reactions catalyzed by Dio1 or Dio3. *Horizontal arrows*, Putative decarboxylation reactions that might represent the first step of TAM biosynthesis. *Dashed lines*, TAMs that have been excluded as precursors of the biosynthesis of 3-T_1AM and T_0AM.

C57BL/6 wild-type mice caused a rapid and pronounced decrease in body temperature (from 36.7 C to ~23.6 C within 120 min) but also toxic effects, such as stiffening of hind limbs, leading to death 24 h after injection (36). Similarly, an N-methylated 3-T_1AM derivative containing a fluorine atom instead of the 4′-OH group caused hypothermia comparably with 3-T_1AM but concomitant toxic effects within 1 wk. Other synthetic 3-T_1AM derivatives that were even as potent as 3-T_1AM at activating heterologously expressed TAAR-1 caused similar hypothermic effects but no toxicity. Taken together, among the compounds studied, those lacking the 4′-OH group exhibited toxicity. Considering that the 4′-OH group represents a target site of sulfation and glucuronidation, it was hypothesized that the lack of 4′-OH might prevent sulfation and glucuronidation of the compounds and thus impair their clearance, which may be a factor in the observed toxicity (36).

B. Sulfation

T_0AM and 3-T_1AM, which are present *in vivo*, but also T_3AM were found to be readily sulfated by distinct human liver sulfotransferase (SULT) isoforms (43). Moreover, 3-T_1AM was sulfated by homogenates of human brain and cardiac tissue, *i.e.*, target tissues of TAM action. These SULT actions might serve to attenuate and thus regulate TAM action (43).

C. Oxidative deamination

3-T_1AM and T_3AM were also found to be subject to oxidative deamination of the ethylamine side chain yielding thyroacetic acids (7). This represents yet another alternate pathway of TH metabolism (44, 45). 3-T_1AM and T_3AM were converted to their respective thyroacetic acids (TA_1 and TA_3) by cultured cells, tissue homogenates, and *in vivo*. These reactions were catalyzed by an amine oxidase activity and were inhibited by the monoamine oxidase and semicarbazide-sensitive amine oxidase inhibitor iproniazid (44).

The biological activity of TA_1 is unknown, so far. Taken together, there is evidence suggesting that sulfation and oxidative deamination are relevant reactions of TAM metabolism. So far, no evidence has become available for 4′-O-methylation or glucuronidation of TAMs.

IX. Cellular Uptake and Transport

3-T_1AM has recently been found to be transported into a variety of cell types via a specific transport mechanism that can be saturated and inhibited (46). To identify the putative 3-T_1AM transporter(s), a systematic large-scale screening analysis of the solute carrier transporter family was performed. No single specific TAM transporter was identified from this screen; however, the sodium- and chloride-independent, pH-dependent, TAM-specific intracellular transport may involve multiple transporters, eight of which were characterized in more detail (46).

The hypothesized receptors, biosynthesizing and/or metabolizing enzymes, and transporters of TAMs are summarized in Table 2.

X. Therapeutic Applications

Although their physiological function remains elusive, 3-T_1AM and T_0AM have already revealed promising therapeutic potential. In one study published so far, 3-T_1AM and T_0AM were used successfully to treat experimentally induced stroke (Fig. 4) (20). In adult C57BL/6 mice injected ip with 50 mg/kg 3-T_1AM or T_0AM either 1 h after or 2 d before the experimental induction of stroke, the infarct volumes were reduced compared with vehicle-treated control mice. The effect was abolished when the TAM-treated mice were placed on a heat pad, indicating that the neuroprotective effect of TAMs required the induction of hypothermia. This study was the first to apply

TABLE 2. Summary of hypothesized receptors, biosynthesizing and/or metabolizing enzymes, and transporters of TAMs

	Experimental model	Mechanism and/or interpretation	Refs.
Receptors			
TAAR-1	Transfected cell lines, isolated rat hearts	Intracellular cAMP accumulation	3, 11, 27, 35
Adra2A	Cell lines, Adra2A-null mice	May modulate effects on metabolism	27, 38
Signal transduction	*Ex vivo* adult rat working heart	Tyrosine phosphorylation and dephosphorylation pathways	11
Biosynthesis and metabolism			
Deiodination (Dio1, Dio2, Dio3)	Cell lines	May be involved in TAM biosynthesis, provided that TAMs are formed by decarboxylation of THs with ≥2 iodine atoms per molecule	14
Oxidative deamination (MAO, SSAO)	Cell lines, homogenates of human thyroids	May serve as precursors in the biosynthesis of thyroacetic acids (*e.g.*, TA_1, triac)	44
Sulfation (SULT1A1, SULT1A3, SULT1E1)	Transfected cell lines, homogenates of human liver, brain, and heart	May deactivate TAMs and thus attenuate TAM effects	43
Transporters			
Hypothesized transporters (SLC7A1, SLC16A7, SLC17A5, SLCO3A1, SLCO4A1, SLC29A2, SLC31A1, SLC43A3)	Transfected cell lines, siRNA screening	Uptake of 3-T_1AM occurs in multiple cell types, uptake properties identified, so far: specific, saturable, inhibitable, pH-dependent, sodium- and chloride- independent	46

MAO, Monoamine oxidase; SSAO, semicarbazide-sensitive amine oxidase; SLC, solute carrier family; siRNA, small interfering RNA.

endogenous compounds to induce hypothermia as a treatment of stroke that might be expected to cause fewer side effects than synthetic compounds (20). Moreover, it reported the first evidence that a cryogen, namely 3-T_1AM, may be prophylactically administered in situations of an anticipated ischemic injury (20).

XI. Thyronamines in Comparison to Other Recently Characterized TH Metabolites and Their Targets

During the last few years, the classical concept of mechanism of TH action has experienced significant expansion and modifications. Apart of the well-documented and most prevailing mechanism of action of the thyromimetic ligand T_3, which acts by modulating the activities of nuclear T_3 receptors (TRs) TRα1, TRβ1, and TRβ2, several other TH-related effects were described and were supported by convincing experimental evidence (Fig. 5) (for recent reviews, see Refs. 47–50).

A. Mitochondrial TRα1 and effects of 3,5,-T_2

A TRα1 variant lacking the N-terminal A/B domains and thus the nuclear location signal is found in mitochondria (Table 3), acting there as a *bona fide* TR transcription factor for mitochondrial circular DNA, which contains T_3-responsive elements (47, 51). Apart from transcription modulation, this TR form is also involved in regulation of the mitochondrial proton gradient and Ca^{-2+} mediated signaling. Mitochondrial biogenesis, ATP generation, and energy metabolism have been known for decades to be regulated by TH status. T_3-mediated effects on various mitochondrial targets and reactions have been established, and strong evidence has been presented that 3,5-T_2 is a powerful TH metabolite tar-

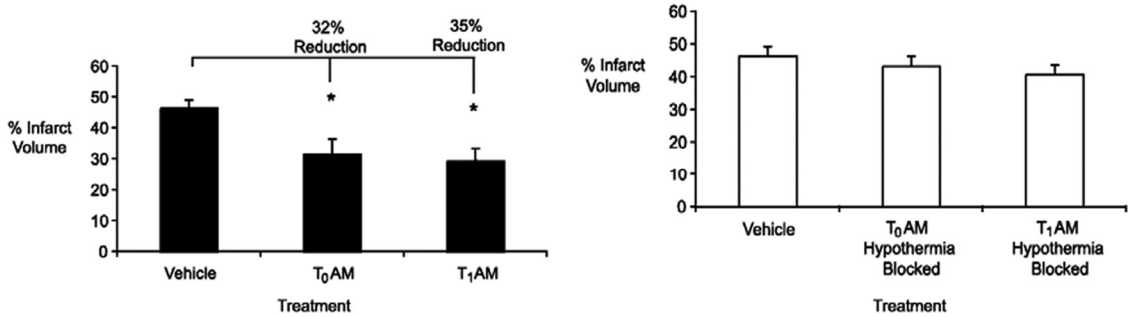


FIG. 4. Infarct volume (percentage of ipsilateral hemisphere) after treatment with 3-T_1AM and T_0AM following middle cerebral artery occlusion (*left*) and under conditions of hypothermia blocked (n = 8 per group). *Error bars* represent SEM. *, $P < 0.05$ compared with vehicle-injected. [Reprinted with permission from K. P. Doyle et al.: *Stroke* 38:2569–2576, 2007 (20). Copyright American Heart Association, Inc.]

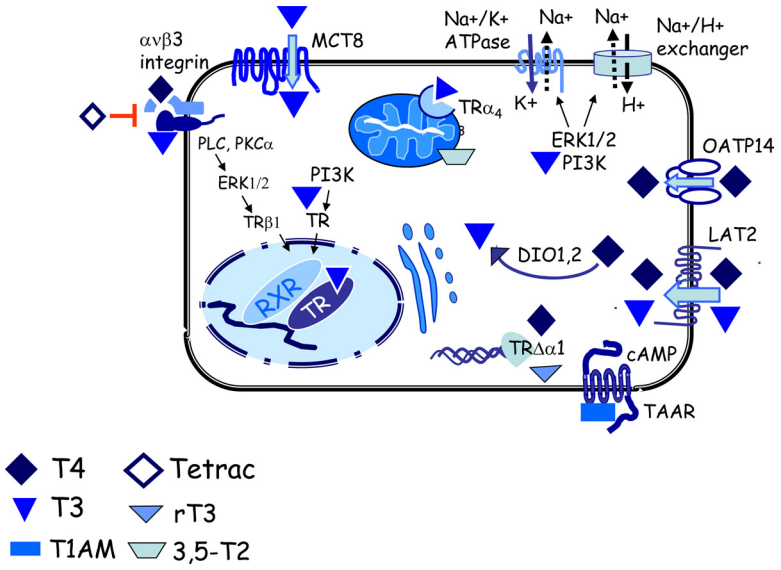


FIG. 5. Schematic overview of effects and target molecules involved in mechanisms of action of thyroid hormones T_4 and T_3 and their metabolites, Tetrac, rT_3, 3,5-T_2, and 3-T_1AM. DIO1,2, Type 1 and type 2 Dio; LAT2, L-type amino acid transporter 2; MCT8, monocarboxylate transporter 8; OATP14, organic anion transporter 14; PKC, protein kinase C; PLC, phospholipase C; RXR, retinoic acid X receptor; TRα43, mitochondrial form of TRα.

geting mitochondrial function (for reviews, see Refs. 48 and 52). 3,5-T_2 in rather high concentrations (25 μg/100 g body weight) rapidly stimulates basal metabolic rate and oxidative capacity in hypothyroid rats, and these effects neither require *de novo* protein biosynthesis nor lead to thyrotoxic cardiac effects, well known for T_3 administration in high concentration. In rodents fed on high-fat diets, 3,5-T_2 also prevents obesity by activating AMP-kinase, which mediates this remarkable stimulation of thermogenesis and mitochondrial fatty acid oxidation in skeletal muscle (52). 3,5-T_2 stimulates flavin-adenin-dinucleotide, reduced form ($FADH_2$)-linked mitochondrial respiratory pathways and alters lipid partitioning by enhancing mitochondrial import and oxidation of long-chain fatty acids. Whether such effects are also observed in euthyroid animals or obese humans remains to be studied. The 3,5-T_2 effects resembling mitochondrial uncoupling in rat muscle and leading to decreased efficiency of energy utilization appear not to be mediated by altered expression of the uncoupling proteins (UCPs) UCP2 or UCP3 (53). Partial uncoupling and decreased H^+/electron stoichiometry had been assigned to binding of 3,5-T_2 to cytochrome c-oxidase subunits, which alters the function of this element of the respiratory chain (48). These effects are distinct from the well-known TRβ-mediated stimulatory effect of T_3 on UCP1 in brown adipose tissue (54), which involves induction of the gene by synergistic action of β3-adrenergic stimulation of cAMP production and induction of Dio2. Modulation of this interaction occurs by additional stimulation of Dio2 by bile acid-mediated activation of the TGR5 (Gpbar-1) plasma membrane GPCR in brown adipocytes and myocytes (55).

B. Plasma membrane-mediated effects of T_4, T_3, and Tetrac

The TH effects described above are obviously opposite to the rapid cryogenic action of 3-T_1AM (37). Rapid plasma membrane-initiated effects of THs and their metabolites have also been observed during development in chicken hepatocytes and various rodent and human cells, including cancer cells (49). Rapid modulation of activity of Na^+,K^+-ATPase, both activation and inhibition depending on cell type and developmental stage (56), has been reported for T_3. However, T_4 and 3,5,-T_2 also exert such effects. Evidence presented so far indicates that this fast activation involves cytosolic kinase signaling cascades such as ERK1/2 and PI3K. Whether these direct effects on Na^+,K^+-ATPase are mediated by TH binding to the plasma membrane αvβ3 integrin receptor or depend on a membrane-associated or cytosolic TR form that is known to interact with PI3K (57) requires further study. Apart from Na^+,K^+-ATPase, the Na^+/H^+ exchanger and the Na^+-dependent amino acid transport system A are rapidly modulated in their activity by T_4, T_3, and to some extent also by rT_3 and 3,5-T_2 (49). The major cellular target initiating these effects appears to be the αvβ3 integrin receptor, which exhibits distinct binding sites for T_4, T_3, and Tetrac. This membrane-initiated signaling requires propagation of the signal by phospholipase C, protein kinase C, Src, or MAPK pathways. The T_4 binding site on the αvβ3 integrin receptor, which is located at the RGD peptide recognition site, shows high T_4 affinity in the range of free T_4 serum concentrations, whereas that of T_3 requires supraphysiological serum concentrations. Surprisingly, Tetrac, the deaminated T_4 metabolite circulating in serum in concentrations comparable to those of T_3, antagonized these effects of T_4 and T_3. Beyond that, Tetrac also blocks integrin-signaling cross talk with other cellular pathways. One final endpoint of action is enhanced angiogenesis, also in the absence of T_4 or T_3 stimulation (49, 50, 58). T_4 stimulation of integrin signaling also increases proliferation of various tumor cells, and again this effect can be efficiently blocked by Tetrac on Tetrac-nanoparticles, which do not enter cells.

Neuronal migration, guided by laminin on glial cells, is affected by rT_3 involving a mechanism associated with polymerization of F-actin, and again this rapid effect ex-

TABLE 3. Overview of targets and effects of natural endogenous and thyroid hormone metabolites as well as synthetic analogs

Tissue/serum concentration	Target structure, receptor, protein	Relevant target organs/cells	Major biological or pharmacological effects	Refs.
3,5,3′,5′-Levo-tetraiodothyronine (L-T_4) (nM to pM)	Plasma membrane ion pores (Na+/K+-ATPase, Na+/H+-exchanger)	Erythrocytes, hepatocytes, several cell types	Rapidly altered signal transduction; control of proliferation and differentiation	49, 56
	$\alpha v\beta 3$ integrin	Several cell types (endothelium, vascular smooth muscle cells, cancer cells, osteoclasts)	Activation of MAPK, angiogenesis cell proliferation, bone metabolism	49, 50
	ERK1/2			
	PI3K			
	F-actin	Developing cerebellum, astrocytes	Neuronal migration under glial guidance	59
3,5,3′-Levo-triiodothyronine (L-T_3) (nM to pM)	TRα, TRβ	Whole body	Permissive and direct effects, basal metabolic rate, growth, differentiation	47
	Plasma membrane receptors ($\alpha v\beta 3$ integrin)	Several cell types including tumor cells	PI3K activation, TRα1 translocation, HIF1α induction; rapidly altered signal transduction; control of proliferation and differentiation	49, 74
3,3′,5′-Levo-triiodothyronine (reverse-T_3, L-rT_3) (pM)	Dio1, Dio3	Many cells	Competing for extrathyroidal T_3 production, Dio inhibitor	50, 75
	Plasma membrane receptors ($\alpha v\beta 3$ integrin)	Several cell types	Activation of several cytosolic kinase pathways	59
	TR$\Delta\alpha$1	Astrocytes	F-actin polymerization	
3,5-Diiodo-L-thyronine (3,5-T_2) (μM to nM)		Liver	Enhanced fatty acid oxidation and carnitine palmitoyl transferase activity	53
	Cytochrome c oxidase subunits	Liver	Mitochondrial uncoupling	48, 53
	AMP-kinase	Skeletal muscle	Enhanced thermogenesis and basal metabolic rate in hypothyroid rats	52
Tetraiodothyroacetic acid (Tetrac)	$\alpha v\beta 3$ integrin (inhibitor)		Angiogenesis	50
Triiodothyroacetic acid (Triac)	TRβ2	Anterior pituitary	TSH suppression	48, 76
		Liver	Cholesterol lowering	66
		Bone	Bone loss	
Triiodothyronamine (Triam)	β-adrenergic receptor	Turkey erythrocytes	Antagonist	4
	Dopamine receptor		Reduced dopamine requirement in intensive care patients	8
TAMs (3-T_1AM/T_0AM) (pmol/g or nM)	See Table 2	See Table 1	See Table 1	
GC-1	TRβ		Partially similiar to T_3	77
	$\alpha v\beta 3$ integrin		Angiogenesis	78
Eprotirome (KB2115)	Hepatic TRβ1	Liver	Decreases atherogenic lipoproteins	71, 72
3,5-Diiodothyropropionic acid (DITPA)	TR	Several cell types	Partially similiar to T_3	
	$\alpha v\beta 3$ integrin		Angiogenesis	50, 61, 79
	TR	Heart	Congestive heart failure	
D-Thyroxine/D-triiodthyronine (D-T_4/D-T_3)	TR	Liver, adipocytes	Lipolysis	68

erted by T_4 and rT_3 but not by T_3 involves $\alpha v\beta 3$ receptor signaling sensitive to RGD interference (47, 59). Rapid $\alpha v\beta 3$ integrin receptor-initiated and RGD sensitive T_4 and T_3 effects have also been reported for bone cells (60) and were related to osteoblast proliferation and T_3-sensitive demineralization of the bone.

The remarkable rapid effects of Tetrac (inhibitory) and T_4 and to a lesser extent T_3 (stimulatory) on angiogenesis during development and in tumors open novel routes toward targeted therapies involving innovative formulations of TH-derived metabolites, *e.g.*, nanoparticular Tetrac (50). Whether endogenous Tetrac is also involved in

the regulation of angiogenesis remains to be studied. Currently, no data are available to suggest a contribution of T_4AM or T_3AM in this context, either as potential intermediates formed from T_4 by decarboxylation and further metabolized to Tetrac or Triac (tiratricol) by oxidative deamination or as TH-derived ligands rapidly activating $\alpha v\beta 3$ integrin receptors. It might well be that various metabolites generated from the amino acid-derived T_4 and T_3, the main secretory products of the thyroid gland, are specifically formed by enzymatic processes in particular tissues during developmental, physiological, or pathophysiological conditions. They may thus expand the repertoire of possibilities to modulate and fine-tune metabolic processes and pathways sensitive to and dependent on thyroid hormone-derived signaling compounds such Tetrac, rT_3, 3,5-T_2, and 3-T_1AM.

XII. Novel Synthetic Analogs of Thyroid Hormones, Specific Ligands, DITPA, and Eprotirome, Specific Ligands for T_3 Receptor Isoforms

Although currently no clinically relevant synthetic analogs of T_4, Tetrac, rT_3, 3,5,-T_2, or 3-T_1AM are administered in (pre-)clinical trials, several T_3 analogs selective for the TR isoforms TRα1, TRβ1, or TRβ2 have been tested at various stages (Table 3). 3,5-Diiodothyroproionic acid (DITPA) has recently received high interest as a potent TR ligand that does not require cellular uptake by the plasma membrane T_3 transporter MCT8 and thus might reach relevant neuronal targets during development in patients with X-linked Allan-Herndon-Dudley syndrome via other TH transporters. Apart from its classical TR agonistic metabolic effects, DITPA has also been used experimentally as a powerful ligand for the rapid plasma membrane-initiated TH effects on angiogenesis, on cardiac remodeling after infarction, and in ischemia models (58, 61, 62).

The successful administration of Triac has been reported for patients with pituitary and general resistance to thyroid hormone due to mutations of TRβ (63–65). Triac and similarly D-T_4 have also been used as cholesterol- and lipid-lowering drugs (66), but long-term administration resulted in adverse effects on bone metabolism and density (Triac) (67) or cardiac problems (D-T_4) (68). Several TRα- or TRβ-selective ligands, both agonists and antagonists, have been developed and tested in cellular and animal experimental models (69, 70). A number of x-ray structures and detailed structural models of the TR ligand binding sites with various ligands have been published, and some compounds such as the T_3 mimetic compound eprotirome (KB2115) have been reported to effectively decrease plasma low-density lipoprotein cholesterol and to stimulate bile acid synthesis in humans (71). Remarkably, these beneficial effects of this TRβ-selective ligand occur without cardiac effects under dosages administered. Even more promising are recent reports on this novel drug demonstrating significant dose-dependent reduction in several atherogenic lipoproteins [low-density lipoprotein cholesterol, apolipoprotein B, Lp(a) lipoprotein] and triglycerides in patients after 12 wk of treatment that was administered in addition to the state-of-the-art statin therapy (72). These alterations occurred without change in serum TSH and T_3 levels and with only minor decreases in total and free T_4 levels, and they did not result in a compensatory increase in serum TSH during the intervention period. No adverse eprotirome effects on cardiac or bone parameters were observed, and free fatty acid levels were not increased. Obviously, it might be very interesting to know whether circulating serum levels of rT_3, Tetrac, 3,5-T_2, or 3-T_1AM are affected by eprotirome administration. More details of eprotirome effects on respiratory coefficient, lipid compartmentation, and subcellular partitioning as well as fatty acid *vs.* glucose oxidation might especially be required to compare the action of this T_3-agonist targeting hepatic TRβ1 with the unique metabolic and cryogenic impact of administration of 3-T_1AM in various animal models.

XIII. Conclusions

3-T_1AM and T_0AM have recently been identified as endogenous signaling molecules exhibiting remarkable *in vivo* effects, such as rapid and profound hypothermia, hyperglycemia, reduction of the RQ, negative chronotropy, and negative inotropy (3, 6). So far, basically all fields of TAM biology appear insufficiently characterized and are in need of further research. It is not known whether further representatives of TAMs apart from 3-T_1AM and T_0AM are present *in vivo* and, if so, which tissue concentrations and half-lives they display. It remains equally elusive whether the aforementioned *in vivo* effects represent the physiological TAM actions or are merely pharmacological in nature. Moreover, the physiological receptor(s) of TAMs remain to be identified unequivocally.

Recent findings, which have not been published in peer-reviewed journals yet, suggest that TAM total serum concentrations are orders of magnitude higher than previously reported, depending on how the sample is processed before analysis (12, 73). Possibly, the strong binding of 3-T_1AM to apolipoprotein B100 has to be taken into account for systematic blood sample workup.

Additional unsettled issues include the sites, pathways, and regulation of TAM biosynthesis, their transport in

blood and across biological membranes, as well as possible pathophysiological and therapeutic implications. Yet, their presence in tissues and serum is established, and so is their interesting and promising pattern of biological effects.

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