Point of VIEW

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A Triple Play for Thyroid Hormone

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ABSTRACT A class of thyroid hormone metabolites has dramatic physiological effects on metabolism and heart rate by still-unknown mechanisms of action. A recent study has discovered that thyronamines can inhibit neuronal reuptake of neurotransmitters and prevent the intracellular transport of monoamines for release. This discovery presents a third signaling pathway for thyroid hormone, expands the role that thyroid plays in the central nervous system, and suggests mechanisms of action for the effects of thyronamine-derived neuromodulators.

Thyroid hormone is a key regulator of metabolism and energetics in the body and is well-known to both chemists and biologists for its unique iodine-containing structure and for the deleterious physiological effects seen when thyroid hormone concentrations are either excessive or deficient. The potential physiological roles for thyroid increased greatly with the discovery that thyronamines, decarboxylated and partially deiodinated metabolites of thyroid hormone, cause a 50% decrease in heart rate and an 8 °C drop in body temperature in mice 30 min after dosing (*1*). Finding molecular mechanisms to explain these physiological effects is key to understanding and potentially exploiting this signaling pathway. On page 390 of this journal, Scanlan and coworkers present evidence that thyronamines inhibit dopamine and norepinephrine transporters, preventing neuronal reuptake of these neurotransmitters, and also inhibit the action of vesicular monoamine transporter 2 (VMAT2), the intracellular transporter responsible for loading secretory vesicles with intracellular monoamines for exocytotic release (*2*). These neuromodulatory effects could help explain the physiological effects of thyronamines and give more evidence on how thyroid hormone acts as a switch for maintaining metabolic homeostasis.

Thyroid hormone is biosynthesized from tyrosine and is originally produced in a tetraiodinated form (T_4) that is then deiodinated to the more potent triiodothyronine (T_3). It binds to the thyroid hormone receptor, a nuclear receptor that regulates transcription of genes containing thyroid response elements in their promoter region (*3, 4*). Through this pathway, T_3 is known to increase heart rate, basal metabolism, and body temperature (*5, 6*). Because of their common biosynthetic precursor, tyrosine, T_3 is also structurally similar to biogenic amines such as dopamine and norepinephrine, if T_3 were decarboxylated to the thyronamine. Although the concept of a thyroid metabolite as a neuromodulator has been mentioned previously (*7*), identifying the nature of the metabolite and its physiological relevance was not achieved. In previous work, a panel of thyronamines was synthesized with different degrees of iodination and screened against various biogenic amine receptors (*1*). A number of compounds were found to bind to an isoform of the trace amine-associated receptor (TAAR1), an orphan G-protein-coupled receptor that had not previously been linked with an endogenous ligand. One of these thyronamines, 3-iodothyronamine (T_1AM), was found to activate the receptor at concentrations that were found to be present in rodent brain. This thyronamine was then shown to cause rapid decrease in heart rate and body temperature in mice that were reversible over time without negative long-term effects on the mice. The effects of T_1AM on heart rate and body temperature were generally the opposite of those of T_3, an indication that T_3 and its iodothyronamine metabolite act to maintain a balance in homeostasis, with T_1AM acting as a quick brake to the more gradual increases seen with T_3.

Although this finding was important, it also raised a number of questions, includ-

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This work suggests that the thyronamine scaffold is capable of selective modulation of the different monoamine transporters.

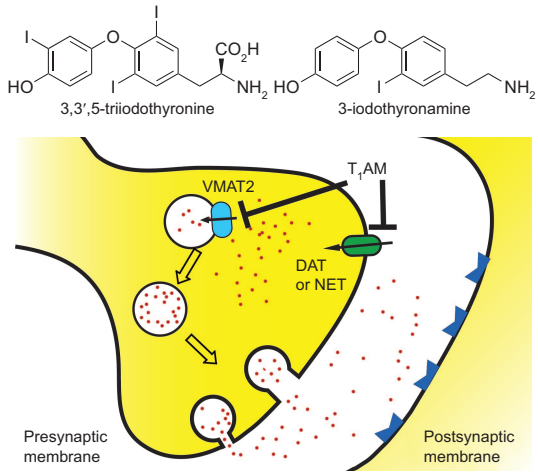

Figure 1. Monoamine release and reuptake by presynaptic neurons. The bioactive form of thyroid hormone T_3 is enzymatically deiodinated and decarboxylated to form thyronamines, including T_1AM. T_1AM inhibits the reuptake of neurotransmitter (red dots) by DAT and NET. T_1AM also inhibits the intracellular transport of monoamines into secretory vesicles by VMAT2. Whether T_1AM can enter cells is unknown.

ing the key question of whether TAAR1 was the mediator of the physiological effects seen in the animal models. The trace amine receptors are a fairly new receptor family whose functions are still poorly understood (*8, 9*). The possibility remains that other receptors that were not tested in the initial screen could also respond to thyronamines. A very relevant family of receptors to consider when testing monoamine action is the monoamine transporter family. At the synapse, neurotransmitters are released from presynaptic neurons through an exocytotic mechanism in which secretory vesicles containing high concentrations of neurotransmitter are shuttled to the plasma membrane for release. After release, neurotransmitters can then bind to receptors on postsynaptic neurons, or they can be inactivated by reuptake into the presynaptic neuron and neighboring neurons by monoamine transporters. There are monoamine transporters specific for dopamine, serotonin, and norepinephrine (named DAT, SERT, and NET, respectively) and an intracellular transporter that packs the monoamines into secretory vesicles for release (VMAT2) (*10, 11*). The monoamine transporters have been vitally important in the development of central nervous system drugs. Most clinically used antidepressants inhibit either the norepinephrine or serotonin transporters (or both), and the dopamine transporter is a major target of amphetamine and cocaine. Various psychostimulants can also inhibit VMAT2, a sign that VMAT2 could be targeted as a strategy to potentially mitigate abuse of these drugs (Figure 1).

Given the structural similarities of thyronamines and the other monoamines, the authors screened a panel of thyronamines against either crude preparations of synaptic termini (known as synaptosomes) from rat brain or mammalian cell lines transfected with specific monoamine transporters. In synaptosomal preparations, T_1AM inhibited the uptake of radiolabeled dopamine and norepinephrine and, to a lesser extent, serotonin but was itself not a substrate for uptake. In transfected cells, the whole panel was tested, and T_1AM was shown to only inhibit the dopamine and norepinephrine transporters and not the serotonin transporter. The authors suggest that the synaptosomal preparations might have SERT-independent mechanisms of serotonin uptake and conclude that T_1AM is not an inhibitor of SERT. Each transporter, including SERT, could be inhibited by at least one member of the panel, although it is interesting to note that the structure–activity relationship for inhibition of each transporter is different. Competition studies showed that T_1AM was a competitive inhibitor of NET but was both a competitive and noncompetitive inhibitor of DAT, an indication of slightly different binding modes in each transporter. Experiments with synaptic vesicle preparations and membranes from transfected mammalian cells showed that T_1AM was also an inhibitor of VMAT2, the intracellular monoamine transporter. It was not a substrate for this transporter, however, and this makes it the first endogenous phenethylamine that is an inhibitor for VMAT2 but not a substrate. Testing the whole panel showed that VMAT2 was more tolerant of substitution on the thyronamine because many of the compounds of the panel could inhibit uptake.

The findings of this paper are significant for a number of reasons. It shows that thyroid hormone metabolites can have significant effects on monoamine neurotransmitter concentrations, both in terms of reuptake of released neurotransmitter and in terms of new neurotransmitter release. This represents a new potential signaling pathway that is ultimately regulated by the thyroid and could shift thinking about thyroid action. Because thyronamines are present in the brain, it raises the intriguing possibility that they could act as some sort of endogenous monoamine reuptake inhibitor. Whether sufficient concentrations of thyronamines can be achieved in the appropriate locations for transporter inhibition to occur is still unknown, but this approach represents a novel neuromodulatory mechanism. This work also suggests that the thyronamine scaffold is capable of selective modulation of the different monoamine transporters, because the structure–activity relationships for each transporter were different.

This paper also raises several interesting questions. Any potential physiological role for thyronamines as reuptake inhibitors will have to address how the thyronamines are generated, how they are localized to synapses, and whether they have their own secretory and reuptake pathways (*12, 13*). This could then add another level of control

if other signaling pathways could regulate these processes. The most relevant question right now is whether transporter inhibition is responsible for the dramatic hypothermia and bradycardia seen when animals are treated with T_1AM. The VMAT2 inhibitor reserpine can elicit responses that are similar to, albeit less dramatic than, T_1AM, but other inhibitors of monoamine transporters such as cocaine and amphetamine generally have the opposite effect on body temperature and heart rate (*14*). In addition, screening with synthetic thyronamine analogues shows a strong correlation between TAAR1 affinity and a compound's potency at lowering body temperature in mice (*15*). TAAR1 has also been identified in cardiac tissue along with other TAAR family isoforms (*16*). As the authors conclude, it is too early to know whether it is thyronamine action at TAAR1 or on monoamine uptake that is responsible for the physiological effects of these thyroid hormone metabolites; however, both pathways could be acting in concert to elicit these effects. The possibility still exists that the effects of the thyronamines on the transporters are indirect, being mediated at least in part by TAAR1. Given the discoveries made so far for this relatively new class of signaling molecules, it is probably safe to say that a more complex picture for thyronamine action and its relationship as a counteragent to the actions of thyroid hormone will emerge. As discoveries in this field have shown thus far, an important component to moving forward in dissecting this complexity will be the development and use of new chemical tools in relevant biological contexts.

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