

## Cardiovascular Consequences of a Mutant Thyroid Hormone Receptor $\alpha 1$

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### Abstract

Thyroid hormone has long been known for its profound direct effects on the cardiovascular system, but its interactions with the autonomic nervous system controlling cardiac activity still remain enigmatic. Recently, mice heterozygous for a mutant thyroid hormone receptor  $\alpha 1$  (TR $\alpha 1$ +/*m*) have been generated and their analysis has provided new insights into the actions of thyroid hormone on the cardiovascular system. The mutant TR $\alpha 1$  caused many symptoms resembling hypothyroidism, such as bradycardia, as well as reduced contraction and delayed relaxation time of isolated cardiomyocytes. While no abnormalities were detected in the autonomic regulation of the basal heart rate using pharmacologic denervation, an impaired adjustment of the autonomic nervous system could be observed in TR $\alpha 1$ +/*m* mice on activity, stress, or increased temperature. The results thus confirm the important role of TR $\alpha 1$  in maintaining the intrinsic properties of the heart and demonstrate a novel role for TR $\alpha 1$  in the adaptations of the autonomic nervous system controlling the heart rate under non-baseline conditions.

### Keywords

Thyroid hormone, heart rate, aporeceptor activity, autonomic nervous system, gene expression, mouse model, cardiac output,  $\beta$ -blocker, timolol, scopolamine, stress, hyperthyroidism, hypothyroidism

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### Thyroid Hormone and its Receptors

The thyroid gland secretes two forms of thyroid hormone: T4 (3,3',5,5'-tetraiodo-L-thyronine, thyroxine) and T3 (3,3',5-triiodo-L-thyronine). While T4 has little biologic activity and is considered a prohormone, T3 is the receptor-active form and exerts most of the thyroid hormone effects.<sup>1</sup>

Tissues can fine-tune their local T3 levels via deiodinases. These enzymes can, for example, activate T4 to T3 by removing an iodine from the outer ring of the hormone (deiodinase type I and II) or inactivate T3 to T2 by inner-ring deiodination (type I and III).<sup>2</sup> Thus, the deiodinases allow certain tissues to regulate their T3 levels autonomously of circulating thyroid hormone.

For a long time it has been assumed that thyroid hormone passes cell membranes due to its hydrophobic properties by diffusion. This view has changed, however, with the recent discovery of specific thyroid hormone transporters, such as MCT8 (monocarboxylate transporter 8) or OATP1C1 (organic anion transport polypeptide 1c1).<sup>3</sup> These transporters can modulate the response of tissues to thyroid hormone depending on their expression level. In the absence of MCT8, for instance, there is no significant uptake of T3 into the mouse brain.<sup>4</sup>

The third level of the cascade that determines the response of a tissue to thyroid hormone is represented by the two nuclear thyroid hormone receptors (TR $\alpha 1$  and TR $\beta$ ). These receptors are encoded by two separate genes located on human chromosomes 17 (TR $\alpha 1$ ) and 3 (TR $\beta$ ), or 11 and 14 in mice. The expression level of these two genes determines the responsiveness of the tissue to thyroid hormone. While TR $\beta$  is predominantly expressed in the liver and auditory system, TR $\alpha 1$  is the major isoform in skeletal muscle, brain and heart.<sup>1</sup> It is still a matter of debate whether genes can be regulated specifically by a single isoform or whether the two receptors can compensate for each other when co-expressed in the same cell.<sup>5</sup>

### Thyroid Hormone and the Heart

Thyroid hormone is well recognized for its profound effects on the cardiovascular system.<sup>6</sup> In patients, hyperthyroidism often leads to tachycardia and cardiac arrhythmias, accompanied by vasorelaxation, decreased systemic vascular resistance, increased blood volume, and systolic hypertension. Hypothyroidism, on the other hand, causes bradycardia, slowed relaxation and reduced cardiac output, together with vasoconstriction and diastolic hypertension.<sup>7,8</sup> Despite these well-known symptoms of hyper- and hypothyroidism, the molecular mechanisms are incompletely understood. Moreover, other factors

such as the age or metabolic condition of the patient strongly modulate the severity of the disorder.

To investigate the cardiovascular actions of thyroid hormone receptors, animal models devoid of one or both thyroid hormone receptors have been generated.<sup>9</sup> Their analyses revealed that TR $\alpha$ 1 represents more than 70 % of thyroid hormone-binding capacity in the heart<sup>6</sup> and is absolutely required for cardiac development.<sup>10</sup> It is also involved in heart rate, relaxation time, and the cardiac response to thyroid hormone.<sup>11</sup> TR $\beta$  is also present in the heart, however, and contributes to the regulation of specific genes, such as cardiac myosin heavy chain.<sup>12</sup> Together with the fact that a TR $\beta$ -selective agonist (GC-1) does not enhance cardiac output to the same extent as T3,<sup>13</sup> it can be concluded that TR $\alpha$ 1 exerts most of the physiological actions of thyroid hormone in the heart.

## Aporeceptor Activity of Thyroid Hormone Receptors

Unlike steroid hormone receptors, thyroid hormone receptors also act in the absence of ligands as aporeceptors. These unliganded receptors (apo-TRs) can, for example, actively suppress the expression of thyroid hormone target genes; an activity that underlies the severe defects observed in untreated congenital hypothyroidism.<sup>1,14,15</sup> The devastating effects of apo-TRs can be impressively demonstrated in the corresponding animal models. Mice born without a functional thyroid gland die within the first three weeks of life,<sup>16–18</sup> while animals lacking all thyroid hormone receptors survive to adulthood.<sup>19</sup> Although thyroid hormone signalling is disrupted in both models, the absence of the hormone is more devastating than the lack of receptors, because the latter also excludes aporeceptor activities.

Similar apo-TR effects can be observed at the molecular level. Hypothyroid mice show a severe repression in HCN2 (potassium/sodium hyperpolarisation-activated cyclic nucleotide-gated channel 2), an important component of the cardiac pacemaker. On the other hand, animals lacking TR $\alpha$ 1 display normal levels and only induction of the gene by T3 is abolished.<sup>20</sup> Taken together, the results clearly demonstrate that unliganded thyroid hormone receptors can have drastic consequences that are not only noticeable at the level of gene regulation but also macroscopically, e.g. in postnatal survival.

These examples also show that mice lacking thyroid hormone receptor genes are not ideal models to analyze the effects of thyroid hormone due to the lack of aporeceptor activity. A second generation of animal models has therefore been developed: mice heterozygous for a point mutation in the ligand-binding domain of the thyroid hormone receptor. This reduces or abolishes affinity to T3 and locks the receptor in a constant aporeceptor state ('receptor-mediated hypothyroidism').

## Consequences of an Unliganded Thyroid Hormone Receptor in the Heart

To analyze the consequences of apo-TR $\alpha$ 1, several strains with a mutant thyroid hormone receptor were recently generated in different laboratories.<sup>21–25</sup> This article will focus on the cardiovascular phenotype of mice with the TR $\alpha$ 1R384C mutation (TR $\alpha$ 1+/m mice), which has been analysed in detail *in vivo* and *in vitro*.<sup>25–27</sup>

As expected from a receptor-mediated hypothyroid state, the TR $\alpha$ 1+/m mice displayed bradycardia. Furthermore, isolated cardiomyocytes of these animals showed a slower decay of intracellular calcium and consequently a prolonged relaxation time. The contraction of the isolated cells was not as pronounced or as fast as that of wildtype cardiomyocytes.<sup>25,27</sup> At the molecular level, the expression of HCN2 was strongly reduced by the mutant TR $\alpha$ 1.<sup>26</sup> As even minor reductions in HCN2 can cause severe bradycardia,<sup>28</sup> the observed reduction is likely to be the underlying molecular cause for the reduced heart rate in TR $\alpha$ 1+/m animals.

Interestingly, although isolated TR $\alpha$ 1+/m cardiomyocytes were more sensitive to  $\beta$ -adrenergic stimulation,<sup>27</sup> the injection of the catecholamine isoprenaline did not raise the heart rate of TR $\alpha$ 1+/m mice to an extent similar to that in wildtype animals.<sup>25</sup> This suggests that an increased basal  $\beta$ -adrenergic stimulation might already exist in TR $\alpha$ 1+/m animals, partially masking the intrinsic defects observed in isolated cardiomyocytes. Thus, the autonomic regulation of cardiac activity in these mutants was further investigated.

## Consequences of a Mutant Thyroid Hormone Receptor For Autonomic Regulation

As the clinical symptoms of hyperthyroidism and excess catecholamine are quite similar, interactions between the two systems have frequently been discussed. The molecular mechanisms remain enigmatic and the observations in patients are contradictory. An increased activity of the sympathetic nervous system is observed in long-term hypothyroidism,<sup>29</sup> but also in patients with untreated Graves' hyperthyroidism.<sup>30,31</sup> Catecholamine levels are usually normal if not lower in hyperthyroidism,<sup>32</sup> however, suggesting that the interactions of thyroid hormone with catecholamine signalling might occur downstream of the receptor.

To identify the molecular mechanisms, different animal models have been analyzed, but the results have not clarified the situation. Some observed a reduced catecholamine turnover in hypothyroid animals<sup>33</sup> and an increased  $\beta$ -adrenergic responsiveness of cardiomyocytes in thyrotoxicosis.<sup>34</sup> Others could not find any evidence for altered catecholamine response in dependence of the thyroid status.<sup>35</sup>

The results obtained from TR $\alpha$ 1+/m mice have provided novel insights. No difference was observed in the mRNA expression of  $\beta$ -adrenergic or muscarinic acetylcholine receptors in the heart between TR $\alpha$ 1+/m mice and wildtype animals, indicating that the receptors themselves are most likely not subject to TR $\alpha$ 1 regulation. Conscious and freely moving TR $\alpha$ 1+/m mice were injected with the cholinergic blocker scopolamine methyl bromide for parasympathetic denervation or with the non-selective  $\beta$ -adrenergic receptor blocker timolol for sympathetic denervation. In these animals, the changes in heart rate were similar to those observed in wildtypes. Thus, these analyses did not provide further evidence for a direct cross-talk between the mutant TR $\alpha$ 1 with the autonomic regulation of heart rate.<sup>26</sup>

During night-time activity periods or when the mice were challenged with a mild stressor, however, adaptation of heart rate did not occur

to the same extent in TR $\alpha$ 1+/m mice as in wildtype animals. This effect was even more pronounced when the mice were reared at 30°C, which reverses the autonomic control of heart rate in rodents from mainly sympathetic to parasympathetic control.<sup>36</sup> While wildtype mice showed the switch in autonomic control at this temperature as expected, TR $\alpha$ 1+/m mice continued to display a predominantly sympathetic regulation of cardiac activity.<sup>26</sup> This suggests that mutant TR $\alpha$ 1 impairs the autonomic adaptations of heart rate upon environmental changes.

In summary, the results obtained in mice with a mutant TR $\alpha$ 1 indicate that the interactions of thyroid hormone and the autonomic regulation of heart rate can be minimal under baseline conditions. When challenged by anesthesia, stress or changes in environmental temperature, however, the effects become more obvious. The findings thus underline the need to reevaluate previous studies where the interactions of thyroid hormone and the autonomic control of heart rate might have been studied under non-baseline conditions, e.g. in anesthetised animals.

### Where are the Patients?

Mutations in TR $\beta$  that abolish or reduce thyroid hormone-binding to the receptor are well-known in humans and lead to thyroid hormone resistance syndrome, which was first described in 1967.<sup>37,38</sup> The phenotype of these patients is characterized by reduced responsiveness of TR $\beta$ -expressing tissues and consequently elevated levels of thyroid hormone due to insufficient suppression of thyrotropin-stimulating hormone (TSH) in the pituitary. The high thyroid hormone levels in turn cause a hyperthyroid state in tissues expressing mainly TR $\alpha$ 1, such as the heart and the brain.

Although the symptoms and penetrance are variable, the mixed hypo- and hyperthyroid phenotype is very characteristic. It is usually accompanied by goiter, tachycardia, and occasionally with psychological disorders, such as learning disability or attention deficit hyperactivity disorder. Currently, more than 300 patient families have been characterized with over 50 different mutations in TR $\beta$ , thus providing a good pool of clinical data that facilitate a reliable diagnosis.

Surprisingly, however, to date no patient has been identified carrying a mutation in TR $\alpha$ 1. Several animal models have been generated to understand the consequences of a mutation in TR $\alpha$ 1 and to predict a human phenotype.<sup>21–25</sup> The task is complicated by the fact that a mutation in the TR $\alpha$ 1 gene can—depending on location—not only lower T3 binding but also abolish interactions of the thyroid hormone receptor with specific co-factors, thus additionally modulating the phenotype. For instance, mice with the TR $\alpha$ 1R384C mutation are metabolically lean, while a TR $\alpha$ 1P398H mutation leads to obesity.<sup>22,25,39</sup> This difference is probably caused by the different abilities of the two mutant receptors to interact with peroxisome proliferator-activated receptors.<sup>40</sup> As the different mutants exhibited no gross abnormalities in thyroid hormone levels as adults, however, it is expected that TR $\alpha$ 1 patients might also display inconspicuous thyroid hormone levels. This could explain why these patients have not been identified yet: the lack of a thyroid phenotype in the anamnesis would most likely lead to the conclusion that thyroid hormone signalling is not affected. The other

common features of the different animal models with a mutant TR $\alpha$ 1 have been used to predict a human phenotype.<sup>41,42</sup>

### Childhood

Young patients with a mutation in TR $\alpha$ 1 are likely to display lower birth weight and delayed physical and mental development. This could include growth retardation, young bone age, impaired speech development, or disturbances in fine motor movement. Moreover, given the important role of TR $\alpha$ 1 in the brain, a mutant TR $\alpha$ 1 could contribute to symptoms such as panic disorder, autism, learning or memory problems, or even epilepsy.<sup>41,42</sup> In terms of cardiovascular function, bradyarrhythmias as well as lower blood pressure might be observed. As TR $\alpha$ 1+/m mutant mice display mild hypothyroidism as young animals, one could expect above-average TSH in the presence of normal T3 or T4 levels.

### Adulthood

As adults, mental impairments such as anxiety, memory problems, or seizures might be present. Given the cardiovascular phenotype of the TR $\alpha$ 1+/m animals, a mild to severe bradycardia could be expected, accompanied by problems adapting the heart rate to environmental changes and stress. This could also include a higher sensitivity to  $\beta$ -blockers. Furthermore, metabolic abnormalities could occur: depending on the position of the mutation, either leanness with problems gaining weight or obesity with disturbed temperature sensation.

Given the fact that TR $\alpha$ 1+/m mice benefit from treatment with thyroid hormone in several aspects, the identification of patients with a mutation in TR $\alpha$ 1 seems an important goal. A close collaboration between scientists and clinicians will be required to identify and characterize the patients with a mutation in TR $\alpha$ 1.

### Conclusions

The transfer of cardiovascular knowledge from animal models to humans is complicated by the fact that the autonomic control of heart rate is mainly under sympathetic control in rodents at room temperature,<sup>43–45</sup> while in humans parasympathetic regulation dominates. The analysis of mice with a mutant TR $\alpha$ 1 has, however, provided new insight into the role of TR $\alpha$ 1 in the cardiovascular system. Besides the expected intrinsic defects—such as reduced contractility, delayed relaxation, and bradycardia—the animals also displayed an impaired autonomic adaptation on stress or alterations in environmental temperature. Although the molecular reason remains to be elucidated, these impairments most likely reside within the central circuits that control cardiovascular activity. Given the prominent role of TR $\alpha$ 1 in the brain, these findings are not surprising.

Taken together, the TR $\alpha$ 1+/m phenotype in mice indicates that thyroid hormone regulates cardiac activity mainly by direct effects on the heart, but also indirectly via the autonomic nervous system. The latter might not be obvious under baseline conditions, but can become relevant for the central adaptations of heart rate to external stimuli, such as stress. These findings should be kept in mind when designing experiments to investigate the interactions of thyroid hormone with the cardiovascular system, especially when they challenge autonomic control of heart rate, such as anesthetics. ■

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