



Given that the liver receives blood draining into its sinusoids from the intestines and mesenteric fat, and that NKT cells patrol liver sinusoids, this discovery raises the intriguing possibility that immune-mediated mechanisms that suppress postprandial glucose production are inherently coupled to those that dampen hepatic immune responses to the gut-derived factors that have been implicated in inflammasome activation and deregulation of neurohumoral mechanisms that control feeding behavior and energy homeostasis. This insight, in turn, may have broad implications for the development of novel strategies to control obesity, insulin resistance, and T2DM.

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A heartfelt response: new thyroid hormone-sensitive neurons in the hypothalamus

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Thyroid hormone is a well-known regulator of metabolic and cardiovascular functions, and signaling through thyroid receptors has differential effects on cells depending on the receptor isoform that they express. In this issue of the JCI, Mittag et al. provide evidence that thyroid hormone receptors are essential for the formation of a population of parvalbuminergic neurons in the anterior hypothalamus, linking, for the first time, impaired thyroid hormone signaling during development to cellular deficits in the hypothalamus. Since this newly discovered cell group is predicted to play a role in regulating cardiovascular function, these findings suggest that developmental hypothyroidism may be the cause of cardiovascular disorders later in life.

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Thyroid hormone plays a key role in regulating many developmental, metabolic, and cardiovascular processes (1, 2). Congenital hypothyroidism, which occurs in 1 in less

than 3,000 births (3), and other thyroid gland disorders are associated with defects in the maturation and function of many tissues and organ systems. It has been a long-standing challenge to decipher the mechanisms by which thyroid hormone regulates such a wide range of cellular processes in so many different tissues. How does the same hormone stimulate the differentiation of an embryonic neuroblast but trigger an entirely different response in an adult liver cell? Thyroid hormone acts through the intracellular thyroid hormone receptor (TR), which belongs to the nuclear receptor family and acts as a ligand-regu-

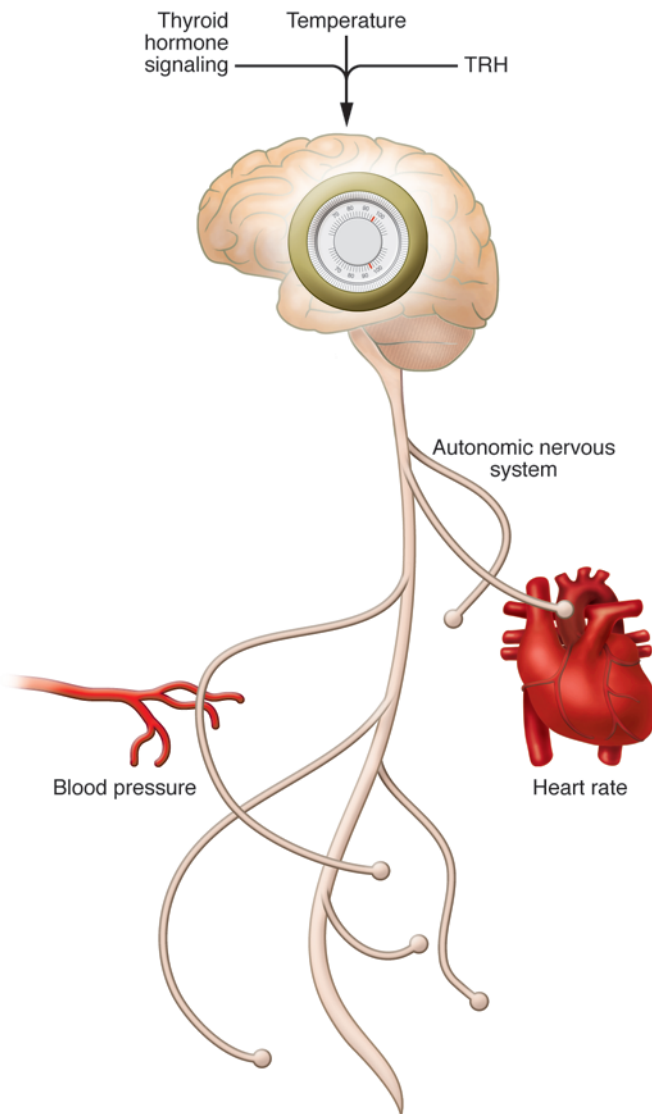


Figure 1

The pv^+ neurons in the AHA are proposed to act as central integrators (indicated by the dial in the brain) that determine the set point for cardiovascular function. These neurons depend on thyroid hormone signaling for proper development and integrate temperature information to regulate cardiovascular parameters via modulating central autonomic outflow. Moreover, the activity of pv^+ neurons is regulated by TRH. The downstream relay stations that transmit signals from the AHA pv^+ neurons to the heart remain unknown at present.

lated transcription factor (4, 5), eliciting cellular responses by binding to and regulating the expression of target genes. The binding of triiodothyronine (T3), the biologically active form of thyroid hormone, induces a conformational change in the TR that leads to a dynamic exchange of associated transcriptional corepressor and coactivator complexes.

T3 acts through three TR isoforms in humans and mice: TR α 1, which is encoded by the *THRA* gene, and TR β 1 and TR β 2, which are encoded by the *THRB* gene. The differential expression of these receptor isoforms determines certain specific cellular functions; for example, TR β 2 in cone photoreceptors is required for color vision (6) and TR α 1 in pyramidal neurons in the hippocampus may influence learning and memory (7). Mutations

in the human *THRA* and *THRB* genes have been associated with a distinct spectrum of symptoms, consistent with the proposal that TR α 1 and TR β isoforms mediate specific functions. *THRA* mutations result in hypothyroid-like defects, with mental and physical retardation but with only marginal abnormalities in serum thyroid hormone levels (8, 9). In contrast, *THRB* mutations result in hyperactivity of the hypothalamic-pituitary-thyroid axis, with elevated levels of thyroid hormones and nonsuppressed thyrotropin as well as a variety of growth and neurological abnormalities (10). Relevant to the study in this issue of the *JCI* by Mittag et al. (11), TR α 1 is expressed widely in the brain, including in the hypothalamus in rodents (12), which is the control center of central autonomic outflow (Figure 1).

Impaired thyroid hormone signaling causes specific defects in the hypothalamus

Until recently, the cardiovascular and metabolic effects of thyroid hormone were thought to be mediated predominantly by TR isoforms expressed by peripheral tissues, including heart, skeletal muscle, or fat (1, 2). However, recent studies suggest that thyroid hormone can also modulate developmental, metabolic, and cardiovascular processes by acting on specific targets in the brain (13–15). The neuronal subpopulations and pathways involved in these CNS-dependent thyroid hormone effects remain poorly defined, particularly as far as the central cardiovascular actions are concerned.

Interestingly, Bochukova et al. (8) recently described a patient who was heterozygous for a *THRA* allele that expressed a dominant-



negative TR α 1 with the ability to inhibit wild-type TR function, causing resistance to thyroid hormone in various target tissues. In addition to other clinical impairments, this patient displayed bradycardia and low blood pressure, a phenotype resembling that observed in mouse models with similar dominant mutations, including the TR α 1 R384C mutant studied by Mittag et al. (11, 15–17). These findings led to the speculation that thyroid hormone–dependent modulation of cardiovascular function may involve central target tissues that are critical for regulating proper autonomic (sympathetic and parasympathetic) outflow (15).

To gain insight into the neuroanatomical basis of these central actions of thyroid hormone, Mittag et al. initially studied TR α 1 R384C mutant mice (11). These mice are heterozygous for the R384C point mutation that reduces T3-binding affinity of TR α 1 by approximately 10 fold. Mittag et al. previously suggested (15) that central autonomic control of cardiovascular responses to stress, activity, or changes in environmental temperature was dysregulated in this mouse model. In the study by Mittag et al., the authors made the interesting observation that the level of parvalbumin (pv) mRNA was reduced by approximately 50% in the hypothalamus of TR α 1 R384C mice (11). Of note, pv⁺ cells are present not only in the hypothalamus but also in many other areas of the brain (18). Immunohistochemistry identified a small population of hypothalamic pv⁺ cells localized in the anterior hypothalamic area (AHA) that had not been described previously. Importantly, in TR α 1 R384C mice, this population of hypothalamic pv⁺ cells was drastically reduced (by ~70%), as compared with that in wild-type control mice, while other hypothalamic pv⁺ cell groups were unchanged. In addition, Mittag et al. found that the selective reduction in the number of AHA pv⁺ cells was due to a developmental defect caused by impaired signaling through both the α 1 and β TR isoforms (11).

To study the possible functional roles of the AHA pv⁺ neurons, Mittag et al. carried out whole-cell patch-clamp recordings, using hypothalamic slices prepared from adult mice expressing GFP in pv⁺ neurons. Interestingly, all analyzed AHA pv⁺ cells were responsive to changes in temperature ranging from 25°C to 40°C, causing either excitation or inhibition in approximately 70% and 30% of the neurons, respectively. Additional studies suggested the involvement of specific ion channels in mediating these opposing

electrophysiological effects. In a similar fashion, treatment with thyrotropin-releasing hormone (TRH) also led to either excitation or inhibition of AHA pv⁺ neurons. However, since no correlation was observed between the nature of the TRH response and the type of temperature sensitivity, the authors postulated the existence of at least four different subpopulations of AHA pv⁺ neurons (11). Future studies aimed at better understanding the properties and potential physiological roles of these various AHA pv⁺ cell populations should be of significant interest.

Potential physiological roles of AHA pv⁺ neurons

To explore the potential physiological relevance of the newly discovered AHA pv⁺ neurons, the authors selectively ablated approximately 40% of the AHA pv⁺ cells in wild-type mice. While the loss of this cell population did not induce major metabolic or behavioral phenotypes, it did lead to pronounced increases in systolic and diastolic blood pressure. Since the serum levels of hormones that are critically involved in blood pressure regulation remained unchanged in these mice, the authors proposed that the observed changes in cardiovascular function might be caused by altered autonomic outflow.

Moreover, following cold exposure (4°C), AHA pv⁺-ablated mice displayed a pronounced increase in heart rate, as compared with that of control mice. This effect was not observed at thermoneutrality (30°C). Mittag et al. also treated AHA pv⁺-ablated mice with N-methylscopolamine, a muscarinic receptor antagonist that interferes with the inhibitory cardiac parasympathetic actions of acetylcholine, and timolol, a β -adrenergic receptor antagonist that blocks the stimulatory sympathetic cardiac effects of norepinephrine (or epinephrine). They showed that the ability of the two antagonists to trigger changes in heart rate was significantly reduced in AHA pv⁺-ablated mice. Taken together, these findings strongly support the concept that AHA pv⁺ neurons play a critical role in the central regulation of cardiovascular function.

Future directions

The study by Mittag et al. has important implications regarding the origin of cellular defects that lead to cardiovascular damage in hypothyroidism. Previous evidence indicated that the heart responds directly to T3 (1). Indeed, cardiomyocytes isolated from TR α 1-deficient mice display abnormal inactivation kinetics of voltage-dependent potassium currents (19). Thus,

a key issue still to be addressed is the relative contribution of central hypothalamic circuits compared with that of peripheral heart responses in regulating overall cardiac function. Obviously, this question is relevant for understanding the bradycardia and low blood pressure phenotypes observed in a human patient carrying the *THRA* mutation (8) and in TR α 1 R384C mice.

Fascinating questions remain to be addressed concerning the mechanism by which thyroid hormone regulates the development of the AHA pv⁺ neurons. It is unknown whether there is a failure to generate these neurons or whether these neurons are initially formed but then succumb to cell death at a later stage. The answer to this question may have implications for our understanding of the pathophysiology of human congenital hypothyroidism. The study by Mittag et al., showing the involvement of TRs in the development of a new subgroup of hypothalamic neurons, complements other recent investigations of the genetic pathways that generate neuronal diversity in several hypothalamic nuclei (20).

Other key unanswered questions that arise from this work (11) concern the neuroanatomical basis for the distinct responses of the postulated four subpopulations of AHA pv⁺ neurons and the nature of the connections through which these neurons regulate cardiac function.

In summary, the authors have expanded our perspective on the role of the hypothalamus in the central control of homeostatic functions. In addition, the findings alert us to the likelihood that thyroid hormone, sometimes considered to be an “old” hormone, will yet be found to mediate new functions. Contemporary techniques provide unprecedented opportunities for dissecting the roles of specific genes in defined cell populations. We may anticipate that continued exploration will identify additional developmental and physiological functions that are under the control of thyroid hormone signaling.

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