Freedom of expression beyond the thyroid: the thyroid-stimulating hormone receptor in the adipocyte

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Abstract

Introduction

The TSH receptor (TSHR) enables thyrocytes to recognize and respond to TSH secreted by pituitary thyrotrophs. It regulates thyrocyte proliferation as well as thyroid hormone production and secretion. Expression of functional TSHR occurs outside of the thyroid gland, and there is ongoing interest in understanding what role TSHR may play in these extra-thyroidal sites. In this regard, adipose tissue has been studied extensively in rodent models and humans. TSHR has been linked to lipolytic responses, as well as to production of adipokines, bioactive factors synthesized and secreted by adipocytes that influence a number of physiological processes such as inflammation and insulin sensitivity. Studies related to TSHR expression in the skeleton and the liver will also be presented.

Conclusion

Evidence continues to accumulate pointing to a role for TSHR beyond the thyroid gland. In adipose tissue, this receptor may be involved in triggering inflammation, insulin resistance, and cardiovascular disease. TSHR has also been implicated in some aspects of bone remodelling and in hepatic cholesterol metabolism. Overall, the studies reviewed here make a persuasive case to consider the impact of TSHR signalling in physiological systems beyond the thyroid gland.

Introduction

The thyroid gland synthesizes thyroxine (T4) and triiodothyronine (T3), and secretes these thyroid hormones to regulate a wide variety of organ and tissues. As shown in figure 1, optimal circulating thyroid hormone levels are achieved through a negative feedback control system, so that a decrease in these hormones is perceived by anterior pituitary thyrocytes. The hypothalamus also participates and directly stimulates thyrocytes via release of thyrotropin (TSH)-releasing hormone. The TSH released into the circulation by pituitary thyrocytes acts on thyrocytes, via their TSH receptors (TSHR), to stimulate thyroid hormone production and maintain normal levels. TSH also has a role in ensuring healthy thyroid gland development¹. Alongside the work that has elucidated the role of TSH in thyroid physiology, there have been related efforts to evaluate whether there may be a role for TSHR outside of the thyroid gland. This review presents what is known about TSHR action in adipose tissue, the most studied extra-thyroidal site, as well as in two other metabolic organs, the bone and liver.

Discussion

The authors have referenced some of their own studies in this review. The protocols for these studies have been approved by the relevant ethics committees related to the institution in which they were performed.

TSHR: structure and signal transduction

The cloning of TSHR in 1989 was a rollicking scientific adventure involving a tight race between several groups, and has been vividly recounted². It is a 764 amino acid (aa) glycoprotein hormone receptor member of the G protein-coupled receptor (GPCR) family. Its molecular structure is complicated, since it can undergo a range of post-translational modifications such as palmitoylation, sulfation, glycosylation, and phosphorylation. Other structural processes involved in TSHR action include cleavage, surface shedding, dimerization, and multimerization³,⁴. The N-terminal large extracellular region (415 aa) contains 11 leucine-rich repeats (LRRs), and is significantly glycosylated. It is joined to the hinge region (130 aa) that links it to the transmembrane domain (349 aa), consisting of 7 transmembrane helices, 3 intracellular loops, 3 extracellular loops, and an intracellular C-terminal region. TSHR can be cleaved within the hinge region, generating a 2 subunit structure, consisting of an extracellular A subunit covalently linked to a transmembrane B-subunit.

The significance of cleavage in vivo remains uncertain⁵. TSHR can couple with several G proteins (often Gs or Gq) to activate a variety of effector signalling pathways. Gs couples to adenylyl cyclase, leading to high intracellular cAMP levels that activate protein kinase A (PKA). Gq binds to and activates phospholipase Cβ, stimulating the production of diacylglycerol and inositol 3-phosphate (IP3) which raises cytosolic calcium, resulting in the activation of protein kinase C (PKC).

TSHR can be selective with respect to signalling effectors. For example, some constitutive activating TSHR mutations only stimulate Gs³. Different TSHR ligands may preferentially favour specific G proteins, e.g. some TSHR stimulating antibodies only activate Gq⁶.

TSH binding and signalling

TSH is a glycoprotein heterodimer composed of a and b subunits. It binds the extracellular N-terminal TSHR domain in a complex way, interacting with LRRs 2-9 at as many as 52 binding sites⁷. Ultimately, this induces

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structural shifts that activate the receptor. Oligomerization of TSHR upon TSH binding was reported, but its cellular significance is not completely understood.

There are differences between endogenous human and bovine TSH, as well as differences between endogenous human versus recombinant human (rh) TSH that alter TSHR signalling. Bovine TSH has a higher affinity to the human TSHR than human TSH due to positively charged residues in the bovine TSH a-subunit that interact with negatively charged residues in TSHR3. This is one of the reasons bovine TSH is routinely used in human cell culture studies.

The degree and type of glycosylation of TSH also alters binding and bioactivity. The TSH a-subunit has 2 asparagine-linked (N-linked) oligosaccharides; and the TSH b-subunit has another. The pattern of human TSH glycosylation can vary, and this results in different downstream signalling being activated e.g. cAMP versus inositol trisphosphate accumulation.

rhTSH differs from endogenous human TSH with respect to extent and type of glycosylation3.

**Introduction to subclinical hypothyroidism**

With mild failure of the thyroid gland, a compensatory and sustained increase in TSH (produced by the pituitary thyrotrophs) serves to maintain normal thyroid hormone production (Figure 1). This condition, known as subclinical hypothyroidism, is distinct from overt hypothyroidism, in which thyroid gland failure progresses and thyroid hormone levels fall despite the elevated TSH levels.

Subclinical hypothyroidism is not uncommon, with a prevalence of 4-20%, yet uncertainty persists regarding the indications for therapeutic intervention9.

Some view the condition as a healthy compensated state that need only be monitored for progression to frank hypothyroidism.

However, accumulating evidence reveals this condition is associated with a higher risk of cardiovascular disease (CVD) and insulin resistance6,9. Experts, acknowledging the evidence gap that exists, have called for randomized clinical trials to thoroughly evaluate the effect of thyroid hormone treatment for this condition10.

**Pathophysiology of subclinical hypothyroidism and its cardiometabolic risk**

The elevated TSH levels in subclinical hypothyroidism restore adequate thyroid hormone production by the thyroid, yet the risk for insulin resistance and CVD rises. This is not clearly explained by traditional factors such as obesity, dyslipidaemia, or hypertension.

Rather, subclinical hypothyroidism appears to be a pro-inflammatory, insulin-resistant state. It is characterized by post-prandial hyperinsulinaemia, impaired endothelium-dependent vasodilation, as well as elevations in C-reactive protein (CRP), interleukin (IL)-6, monocyte chemoattractant protein 1 (MCP-1; also known as chemokine C-C motif ligand-2 or CCL2), and non-esterified fatty acids (NEFA)9,11,12,13,14.

We have hypothesized (Figure 1) that elevated TSH levels, while compensating for the mild thyroid gland failure, may act on adipocytes in an extra-thyroidal manner15,16,17. This in turn may lead to elevations in circulating NEFA and pro-inflammatory adipokines that predispose to the development of insulin resistance and atherosclerosis18.

**Effect of acute TSH elevation on metabolic and inflammatory effects**

An alternate approach to examine the effect of high TSH levels in vivo is based on the rhTSH stimulation protocol. This involves patients with a history of thyroid cancer treated by thyroidectomy and radioablation i.e. no remaining thyroid gland, who are chronically treated with thyroid hormone. Periodically, they undergo rhTSH stimulation for surveillance of recurrent disease, involving baseline blood work, followed by rhTSH (0.9 mg i.m. x two daily doses), and blood sampling over five days to measure thyroglobulin, which serves as a biochemical marker of thyroid cancer.

Figure 1: The adipocyte as an extra-thyroidal target of TSH. Under healthy circumstances, a negative feedback regulatory system ensures normal thyroid hormones levels. With mild thyroid gland failure (subclinical hypothyroidism), elevated levels of TSH are required to maintain thyroid function, and these higher TSH levels may accelerate adipocyte lipolysis and increase pro-inflammatory adipokine release. See text for details.
Adipocytes as an extra-thyroidal site of TSHR expression and function

It is under-recognized that adipocytes express TSHR. Nobel prize-winning studies from the 1960’s on G proteins by Rodbell included adipocyte lipolysis studies using a variety of ligands including catecholamines and TSH. This work was somewhat forgotten, but in the mid-1990’s, TSH was cloned from rodent adipocytes in 1995. Along with other studies using updated methodologies, awareness of the adipocyte TSHR was re-established.

Understanding how TSH might function in adipocytes is a priority, since this could explain the higher risk for insulin resistance and CVD in patients with subclinical hypothyroidism, a condition marked by elevated serum TSH levels.

TSH and adipocyte lipolysis

Figure 2 shows the release of fatty acids from the perilipin-coated lipid droplet within adipocytes involves the sequential and regulated activation of adipose triglyceride lipase to metabolize triacylglycerol to diacylglycerol, followed by the conversion of diacylglycerol to monoaoylglycerol by hormone-sensitive lipase (HSL). The final step to the remaining fatty acid group is via monoaoylglycerol lipase, an abundant constitutively active enzyme. Phosphorylation of perilipin and HSL are essential for effective lipolysis to occur.

Mice with an adipocyte-specific TSHR deletion, based on the P2 promoter, are unable to activate lipolysis in response to TSH, and develop adipocyte hypertrophy.

TSH stimulates lipolysis in human adipocytes in culture, with neonatal tissue exhibiting a higher sensitivity and supports the idea that the rise in TSH levels in human neonates might be a lipolytic trigger to meet nutritional demands at that time. An exercise study examining adults with subclinical hypothyroidism found elevations in basal levels of NEFA and glycerol. Acute rhTSH treatment of patients previously treated for thyroid cancer also led to an elevation in serum NEFA levels.

Signalling pathways triggered by TSH in adipocytes to stimulate lipolysis have been studied using cell culture models. A rise in cAMP was noted in response to TSH using neonatal adipose tissue. TSH treatment of adult differentiated human adipocytes activates the cAMP target, protein kinase A (PKA).

Phosphorylation of HSL and perilipin occur (both are direct targets of PKA), and the PKA inhibitor H89 blocks these events.

Further studies on the regulation of lipolysis by TSH and PKA revealed that TSH activates conventional PKC (cPKC) in human differentiated adipocytes, upstream of PKA. The cPKC inhibitor Gö6976 (targets PKC isoforms PKCα and βI) reduces TSH-stimulated PKA activity, perilipin phosphorylation, and lipolysis. However, there is no information on the molecular mechanisms by which cPKC influences PKA.

TSH and pro-inflammatory cytokines

Cytokines are elevated in patients with elevated TSH levels associated with subclinical hypothyroidism; the list includes CRP, interleukin (IL)-6, monocyte chemoattractant protein 1 (MCP-1; also known as chemokine C-C motif ligand-2 or CCL2). In addition, rhTSH administration in treated thyroid cancer patients raises the levels of several pro-inflammatory cytokines and pro-atherogenic factors, including IL-6, TNFa, lipoperoxide,
leptin, sICAM-1, sE-selectin, sPselectin, sCD40L, and total 8-isoprostanoglandin F2α. The TSH target cell responsible for these alterations in such in vivo studies cannot be precisely identified. In addition to adipocyte TSHR expression, the presence of TSHR has been reported in vascular endothelial and smooth muscle cells.

To focus on more specific details about the pro-inflammatory action of TSH on adipocytes, differentiated adipocytes in culture were studied. Using TSH-treated 3T3-L1 adipocytes and the PKA inhibitor H89, it was found that IL-6 mRNA expression and protein release into the medium depended on PKA. In contrast, studies that followed using human differentiated adipocytes found that TSH-stimulated IL-6 release depended on the IKKβ/NF-κB pathway, and this pathway was not inhibited by H89. Further studies showed TSH activates PKCd and stimulates NADPH oxidase. Interfering with the action of NADPH oxidase, either with diphenyleneiodonium (inhibits oxidases) or N-acetyl cysteine (scavenges reactive oxygen species) reduced the ability of TSH to activate IKKβ.

TSH signalling studies on human differentiated adipocytes identified MCP-1 as a TSH-responsive adipokine. The increase in mRNA expression and protein release in response to TSH were blocked by either PKA inhibitor H89 or IKKβ inhibitor sc514. The adipocyte factors vascular endothelial growth factor, retinal binding protein 4, and adiponectin were examined as potential targets of TSH, but their expression was not affected by TSH treatment of adipocytes.

Figure 2 summarizes the TSH signalling pathways that may be involved in regulating IL-6 and MCP-1 in the adipocyte.

**TSHR in the skeleton and the liver**

TSHR has also been implicated in the function of other extra-thyroidal tissues, including the bone and liver. TSHR is expressed by osteoblasts and osteoclasts, but its role remains unclear. Initial work proposed that the osteoporosis associated with hyperthyroid states is not only due to excess action of thyroid hormones, but that the loss of TSH signalling due to suppressed TSH levels also contributes to the osteoporosis. A recent review notes a degree of controversy amongst investigators on this topic.

TSHR is expressed at the mRNA and protein level in hepatocytes in rodents and humans. Treatment of a human hepatocyte cell line with either TSH or Graves’ disease immunoglobulins (bind to and stimulate TSHR) raised cAMP production.

Further studies demonstrated that TSH-stimulated hepatocytes up-regulate expression of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase, acting via a PKA-cAMP response element binding protein pathway. The authors speculated that TSH action on the hepatocyte in this manner might contribute to hypercholesterolemia and elevated CRP levels, features that are associated with subclinical hypothyroidism.

**Conclusion**

TSHR expression occurs in several organs and tissues outside of the thyroid, but the evidence is still insufficient to clearly support a role in normal physiology or in pathophysiologic states. Adipose tissue TSHR expression and action has received relatively more attention than other extra-thyroidal sites.

A combination of adipose cell culture studies and in vivo investigations suggest the possibility that this receptor may be involved in lipolytic and pro-inflammatory responses. Learning more about how TSH disturbs healthy adipocyte function may offer insights into the clinical association of elevated TSH levels in subclinical hypothyroidism with insulin resistance and CVD.

**Abbreviations list**

aa, amino acid; AC, adenyl cyclase; ATGL, adipose triglyceride lipase; CRP, C reactive protein; CVD, cardiovascular disease; DG, diacylglycerol; FABP, fatty acid binding protein; HSL, hormone-sensitive lipase, IKK, inhibitor of B kinase; MG, monoaoylglycerol; MGL, monoaoylglycerol lipase; IL, interleukin; LRR, leucine rich repeat; MCP, monocyte chemoattractant protein; NEFA, non-esterified fatty acid; NF-κB, nuclear factor B; PLC, phospholipase C; PKA, protein kinase A; PKC, protein kinase C; rh, recombinant human; ROS, reactive oxygen species; TG, triacylglycerol; TSH, thyroid-stimulating hormone; TSHR, TSH receptor

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**References**


Review

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