Small molecule TSHR agonists and antagonists

Abstract

TSH activates the TSH receptor (TSHR) thereby stimulating the function of thyroid follicular cells (thyrocytes) leading to biosynthesis and secretion of thyroid hormones. Because TSHR is involved in several thyroid pathologies, there is a strong rationale for the design of small molecule “drug-like” ligands. Recombinant human TSH (rhTSH, Thyrogen®) has been used in the follow-up of patients with thyroid cancer to increase the sensitivity for detection of recurrence or metastasis. rhTSH is difficult to produce and must be administered by injection. We developed a small molecule TSHR agonist that is a full agonist at TSHR. Importantly for its clinical potential, this agonist elevated serum thyroxine and stimulated thyroidal radioiodide uptake in mice after its absorption from the gastrointestinal tract following oral administration. Graves’ disease (GD) is caused by persistent, unregulated stimulation of thyrocytes by thyroid-stimulating antibodies (TSAbs) that activate TSHR. We identified the first small molecule TSHR antagonists that inhibited TSH- and TSAb-stimulated signalling in primary cultures of human thyrocytes. Our results provide proof-of-principle for effectiveness of small molecule agonists or antagonists for TSHR. We suggest that these small molecule ligands are lead compounds for the development of higher potency ligands that can be used as probes of TSHR biology with therapeutic potential.

Résumé

L’activation du récepteur de TSH (RTSH) stimule le fonctionnement des cellules vésiculaires de la thyroïde (thyréocytes), conduisant à la biosynthèse et à la libération des hormones thyroïdiennes. Comme le récepteur de la TSH est impliqué dans de nombreuses pathologies thyroïdiennes, il y a une forte motivation pour le développement de petites molécules capables d’intervenir en tant que médicaments ligands. La TSH humaine recombinante (rhTSH, Thyrogen®) a été utilisée dans la surveillance des patients traités pour cancer thyroïdien afin de sensibiliser la détection des récidives et métastases. La rhTSH est de production difficile et doit être administrée par voie injectable. Une petite molécule agoniste de RTSH pourrait procurer le même bénéfice, mais avec une plus grande commodité en administration orale. Nous avons développé une petite molécule ligand qui constitue un agoniste complet de RTSH. Confortant son potentiel d’utilisation en pratique clinique, chez la souris, l’agoniste accroît la thyroxinémie et le captage thyroïdien après absorption intestinale de la médication administrée par voie orale. La maladie de Basedow est liée à la stimulation continue, dérégulée des thyréocytes par des anticorps stimulants, activant RTSH (TSAb). Nous avons identifié la première petite molécule antagoniste de la signalisation au sein de cultures primaires de thyréocytes humains stimulée par TSH ou TSAb. Nos résultats démontrent l’efficacité des petites molécules agonistes ou antagonistes de RTSH. On suggère que ces petites molécules ligands constituent des composés pionniers pour la mise au point de ligands à forte puissance, capables de promouvoir l’approche biologique et thérapeutique de RTSH.

Mots clés : Thyroïde ; Cancer thyroïdien ; Maladie de Basedow ; Récepteur de TSH ; Petites molécules ligands

Keywords: Thyroid; Thyroid cancer; Graves’ disease; TSH receptor; Small molecule ligands

The biologic role of thyroid-stimulating hormone (TSH, thyrotropin) as an activator (agonist) of the TSH receptor (TSHR) in the hypothalamic-pituitary-thyroid axis is well known. Circulating TSH activates TSHR thereby stimulating the function
of thyroid follicular cells (thyrocytes) leading, in particular, to increases in size and number of thyrocytes, and biosynthesis and secretion of thyroid hormones.

Several thyroid pathologies are associated with the TSHR [1], and these diseases provide a strong argument for the design of agonists and antagonists for the TSHR. A number of different potential TSHR ligands have been reported including recombinant human TSH (rhTSH), TSH analogs and antibodies [2]. Our studies have focused on the development of small molecule ligands—agonists and antagonists—that are generally much more easily employed as probes and drugs compared to peptides or proteins. They are synthesized chemically, can be produced in large quantities and can typically be given orally because they are not degraded within and can be absorbed from the gastrointestinal tract.

The incidence of thyroid cancer has progressively increased over the last several years. Since most cases of thyroid cancer are diagnosed in patients between the ages of 20 and 54, patients will have decades of follow-up because it appears that thyroid cancer patients benefit from regular monitoring. For the last decade, rhTSH (Thyrogen®, Genzyme) has been used in this follow-up to increase the sensitivity for detection of recurrent or metastatic thyroid cancer [3]. In addition, rhTSH was recently approved by the Food and Drug Administration for a supplemental indication to improve radiiodine ablation of thyroid remnants after surgical thyroidectomy in patients with thyroid cancer [4]. rhTSH, which is a heterodimeric 30 kDa glycoprotein, is difficult to produce and must be administered by injection, which limits its clinical use. A small molecule TSHR agonist would be worthwhile because it could produce the same beneficial effects as rhTSH but with greater ease of oral administration and therefore be available for use in a larger patient population.

Quantitative high-throughput screening of a library of 73,000 compounds and subsequent chemical modification of the identified lead compound led to the development of a small molecule agonist that is highly selective for human TSHR versus the closely related glycoprotein hormone receptors for luteinizing hormone/chorionic gonadotropin and follicle-stimulating hormone [5]. This small molecule ligand is a full agonist at TSHR by comparison to a maximally effective concentration of TSH with an EC₅₀ of 40 nM and interacts with the receptor’s serpentine domain. In contrast, TSH binds to the extracellular domain of the TSHR. In primary cultures of human thyrocytes, the agonist increases mRNA levels for thyroglobulin, thyroperoxidase, sodium-iodide symporter and deiodinase type 2. More importantly for its clinical potential, this agonist elevated serum thyroid hormone/chorionic gonadotropin and follicle-stimulating hormone [5]. This small molecule ligand is a full agonist at TSHR by comparison to a maximally effective concentration of TSH with an EC₅₀ of 40 nM and interacts with the receptor’s serpentine domain. In contrast, TSH binds to the extracellular domain of the TSHR. In primary cultures of human thyrocytes, the agonist increases mRNA levels for thyroglobulin, thyroperoxidase, sodium-iodide symporter and deiodinase type 2. More importantly for its clinical potential, this agonist elevated serum thyroid hormone/chorionic gonadotropin and follicle-stimulating hormone [5]. This small molecule ligand is a full agonist at TSHR by comparison to a maximally effective concentration of TSH with an EC₅₀ of 40 nM and interacts with the receptor’s serpentine domain. In contrast, TSH binds to the extracellular domain of the TSHR. In primary cultures of human thyrocytes, the agonist increases mRNA levels for thyroglobulin, thyroperoxidase, sodium-iodide symporter and deiodinase type 2.

Graves’ disease (GD) is caused by persistent, unregulated stimulation of thyroid cells by thyroid-stimulating antibodies (TSAbs) that activate the TSHR. TSAbs, like TSH, bind primarily to the large amino-terminal ectodomain of TSHR. We identified the first small molecule TSHR antagonist, which inhibited TSH- and TSAb-stimulated signalling [7], and the first TSHR inverse agonists [8,9], which are antagonists that inhibit basal (or constitutive or agonist-independent) TSHR signalling in addition to TSH- or TSAb-stimulated signalling. TSHR is one of a minority of G protein-coupled receptors that exhibit easily measurable basal signalling activity in vitro [10].

These small molecule allosteric antagonists likely bind to the transmembrane pocket and inhibit signalling by preventing the TSHR from undergoing the conformational changes necessary for activation rather than by interfering with TSH or TSAb binding. To be used as therapy for GD, it would be important that an antagonist inhibits TSHR activation by the majority of TSAbs. Noteworthy, in primary cultures of human thyrocytes, the inverse agonist inhibited cAMP production by average 39% by all thirty GD sera tested [9]. Another possible clinical application of an antagonist beyond treatment of the hyperthyroidism of GD could be alleviating the symptoms of Graves’ ophthalmopathy. The inverse agonist decreased the expression of the mRNAs for the genes thyroperoxidase, TSHR, thyroglobulin and sodium-iodide symporter in the absence of any agonist in primary cultures of human thyrocytes [8]. This observation supports the idea that an inverse agonist could be used to suppress TSH-independent signalling in humans. In addition to GD there are at least two patient groups in which inverse agonists could be used therapeutically. Patients with non-autoimmune hyperthyroidism caused by constitutively activating mutations (CAMs), especially germline mutations, are an obvious group in whom inverse agonists could be effective. We have shown that the inverse TSHR agonist inhibits basal signalling by disease-associated CAMs expressed in cells in tissue culture [8]. A larger patient group in which these drugs would be useful are patients with recurrent or metastatic thyroid cancer who are receiving thyroid hormones for TSH suppression but who may still have their cancer cells stimulated to proliferate and metastasise because of the agonist-independent signalling of TSHR.

Our results provide proof-of-principle for effectiveness of small molecule agonists and antagonists including inverse agonists for TSHR. Chemical optimisation of these small molecule ligands supported by molecular modelling will be performed in an effort to further improve their potencies and therapeutic potential. It is anticipated that a complementary approach using rational modifications of these new ligands will produce more potent molecules for testing in animal models and for future clinical development.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgments

This work was supported by the Intramural Research Programs of the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health.
References


