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Small molecule TSHR: Agonists and antagonists

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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. A small molecule TSHR agonist could produce the same beneficial effects as rhTSH but with greater ease of oral administration. We developed a small molecule ligand 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 and 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.

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 radioiodine 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 thyroxine and stimulated radioiodide uptake by the mouse thyroid gland after its absorption from the gastrointestinal tract following administration by esophageal gavage [6]. These data show that this small molecule agonist can be used as a probe of the molecular mechanism of TSHR activation and to study TSHR function in cells in culture and in an animal model, and may be a drug candidate to be used in patients with thyroid cancer.

Graves' disease (GD) is caused by persistent, unregulated stimulation of thyroid cells by thyroid-stimulating antibodies (TSAbs) that activate the TSHR. TSBAs, like TSH, bind primarily to the large amino-terminal ectodomain of TSHR. We identified the first small molecule TSHR antagonist, which inhibited TSH- and TSBAb-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 TSBAb-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 TSBAb binding. To be used as therapy for GD, it would be important that an antagonist inhibits TSHR activation by the majority of TSBAs. 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. 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.

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