

## Update in TSH Receptor Agonists and Antagonists

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The physiological role of the TSH receptor (TSHR) as a major regulator of thyroid function is well understood, but TSHRs are also expressed in multiple normal extrathyroidal tissues, and the physiological roles of TSHRs in these tissues are unclear. Moreover, TSHRs play a major role in several pathological conditions including hyperthyroidism, hypothyroidism, and thyroid tumors. Small molecule, "drug-like" TSHR agonists, neutral antagonists, and inverse agonists may be useful as probes of TSHR function in extrathyroidal tissues and as leads to develop drugs for several diseases of the thyroid. In this Update, we review the most recent findings regarding the development and use of these small molecule TSHR ligands. (*J Clin Endocrinol Metab* 97: 4287–4292, 2012)

The physiological role of the TSH receptor (TSHR)<sup>1</sup> as a major regulator of thyroid function by controlling the size and number of thyroid cells (thyrocytes) and their synthesis and secretion of thyroid hormones is well understood. Importantly, TSHRs are also expressed in multiple normal extrathyroidal tissues including fat, fibroblasts, bone, brain, kidney, testis, and cells of the immune system (1), but the physiological roles of TSHRs in these tissues are unclear. A role for TSHR in maintaining normal bone homeostasis has been proposed (2), but this conclusion has been challenged (3).

TSHRs play a major role in several pathological conditions, including hyperthyroidism, hypothyroidism, and thyroid tumors. With regard to the number of patients affected, the role of TSHRs in Graves' disease (GD), an autoimmune disease (prevalence is about 1% of the U.S. population), is most important (4). In GD, TSHRs on thyroid cells are continuously activated by circulating thyroid stimulatory antibodies (TSABs) causing overproduction of thyroid hormones leading to hyperthyroidism (5). In addition, it is thought that TSHRs on preadipocytes (fibroblasts)/adipocytes in the orbital space of patients with GD are activated to cause cell proliferation and increased deposition of extracellular matrix leading to expansion of orbital tissue causing Graves' orbitopathy/ophthalmopathy (GO) (6). In a much less common form of nonautoimmune hyperthyroidism, TSHRs on

thyroid cells are mutated receptors that are active in the absence of TSH or TSAB, and these mutated TSHRs signal constitutively (constantly) leading to hyperthyroidism (7). If these mutations are in germline cells, a symmetric goiter (as found in GD) is formed, but if the mutation occurred in a somatic cell, an adenoma forms.

A role for TSHR in some forms of hypothyroidism has also been established. Loss-of-function TSHR mutations in which the binding or signaling functions of the receptor are diminished will lead to a state in which the thyroid gland is underdeveloped and unable to produce sufficient thyroid hormone to maintain a euthyroid state (8). A similar deficiency of thyroid hormone production can be produced by a decrease in TSH production or production of a TSH that exhibits a deficiency in biological activity (9). In these conditions, TSHR still plays a central role.

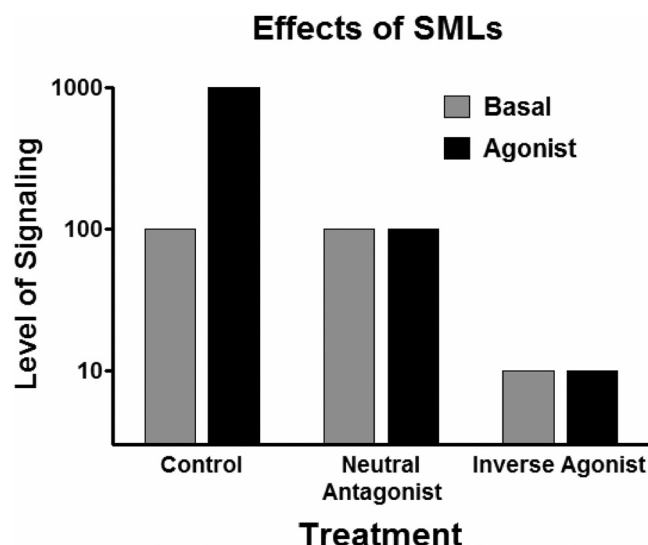
Lastly, TSHR may play an important role in the pathogenesis of thyroid cancer. This idea is based on several findings, including that TSHR expression is required for thyroid tumor initiation in a mouse model (10) and correlation between higher serum concentrations of TSH and greater risk of the genesis of thyroid cancer in patients with nodular goiter (11). TSHR plays a central role in the follow-up of patients with thyroid cancer. Recombinant human TSH (rhTSH) has been used to great advantage in the follow-up of

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<sup>1</sup> We use the abbreviation TSHR to represent the human TSHR and add the species if the receptor is other than human.

Abbreviations: CG, Chorionic gonadotropin; GD, Graves' disease; GO, Graves' orbitopathy/ophthalmopathy; GOF, Graves' orbital fibroblast; GPCR, G protein-coupled receptor; GPHR, glycoprotein hormone receptor; rhTSH, recombinant human TSH; SML, small molecule ligand; TSAB, thyroid stimulatory antibody; TSHR, TSH receptor.



**FIG. 1.** Effects on receptor signaling by agonists, neutral antagonists, and inverse agonists. Control illustrates signaling in the absence of neutral antagonists or inverse agonists. Basal signaling, which represents agonist-independent (or constitutive) signaling, is set as 100. (TSHR is a receptor that exhibits significant basal signaling activity.) Antagonist is a general term that includes neutral antagonists and inverse agonists, which have also been named negative antagonists.

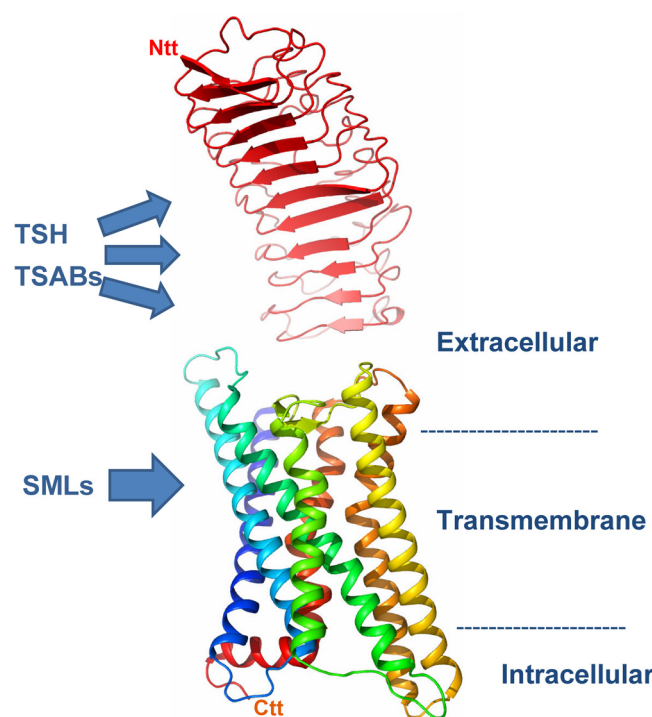
thyroid cancer patients (12, 13). And TSHR activation by rhTSH is being evaluated as an adjunct in the treatment of patients with thyroid cancer and nodular goiter (14, 15).

Small molecule, “drug-like” TSHR agonists (ligands that activate receptors), neutral antagonists (ligands that inhibit receptor activation by agonists), and inverse agonists (ligands that inhibit receptor activation by agonists and additionally inhibit agonist-independent, also termed basal or constitutive, signaling) (Fig. 1) may be useful as probes of TSHR function in extrathyroidal tissues and as leads to develop drugs for several thyroid diseases. With regard to clinical usefulness, agonists could be used to develop drugs that could be used in place of rhTSH in patients with thyroid cancer or benign multinodular nontoxic goiter. Neutral antagonists could be used as leads to develop drugs to antagonize TSAb activation of TSHR in patients with GO. Inverse agonists could be used as leads to develop drugs to inhibit constitutive TSHR signaling in patients with residual thyroid cancer and thereby treat them more effectively than by TSH suppression alone. Furthermore, patients with non-autoimmune hyperthyroidism caused by constitutively activating germline mutations could benefit from an inverse agonist. Indeed, antibodies with these various characteristics have been proposed as therapies for several of these diseases (16).

Because a recent article has reviewed the topic of TSHR antibodies (16), we will limit this Update to small molecule, “drug-like” ligands (SMLs)<sup>2</sup> of TSHR and to findings published during the last 2 yr.

## SML Binding to TSHR

TSHR is a member of a small subfamily of related glycoprotein hormone receptors (GPHRs) that includes receptors for LH/chorionic gonadotropin (CG) and FSH, within class A of the large family of G protein-coupled (seven transmembrane-spanning) receptors (GPCRs). GPCRs comprise the single largest family of signaling molecules found in the human genome. GPHRs are different than other members of class A GPCRs by having a very large amino-terminal ectodomain (Fig. 2). It has been known for a number of years that TSH and TSABs bind primarily to TSHR via interactions with regions within the large ectodomain (17). By contrast, we showed that SMLs bind in a pocket within the seven helical transmembrane bundle of TSHR (18, 19). For receptor functionality, this difference is important because TSHRs with mutations in their ectodomain that do not bind TSH or TSABs, and thereby are not stimulated by them, can be activated by SMLs (see below). Moreover, because TSABs bind to the extracellular domain, whereas SML antagonists bind within the transmembrane domain, the likelihood is high



**FIG. 2.** Domains of binding to TSHR of TSH, TSABs, and SMLs. TSHR, like the receptors for FSH and LH, has a large amino-terminal ectodomain that protrudes from the surface of the cells. TSH and TSABs bind primarily to the TSHR ectodomain. By contrast, SMLs bind to a pocket within the transmembrane domain, which contains the seven  $\alpha$ -helical bundles that are characteristic of GPCRs. Ectodomain, Red. Ntt shows the amino terminus of TSHR. Transmembrane domain: Individual helices are ribbons in different colors. The terminal part of the intracellular carboxyl tail (Ctt) is not shown. This model was kindly provided by Gunnar Kleinau, Ph.D., Charité Campus Virchow Klinikum, Berlin, Germany.

<sup>2</sup> We define SMLs as nonpeptidic compounds of molecular weight less than 750 Da.

that signal transduction initiated by the majority of TSABs can be blocked by SML antagonists (see below). Signal transduction is accomplished by interactions between the intracellular surface of the transmembrane helices and the carboxyl-terminal tail of TSHR with coupling proteins, such as G proteins and arrestins.

## SML TSHR Agonists

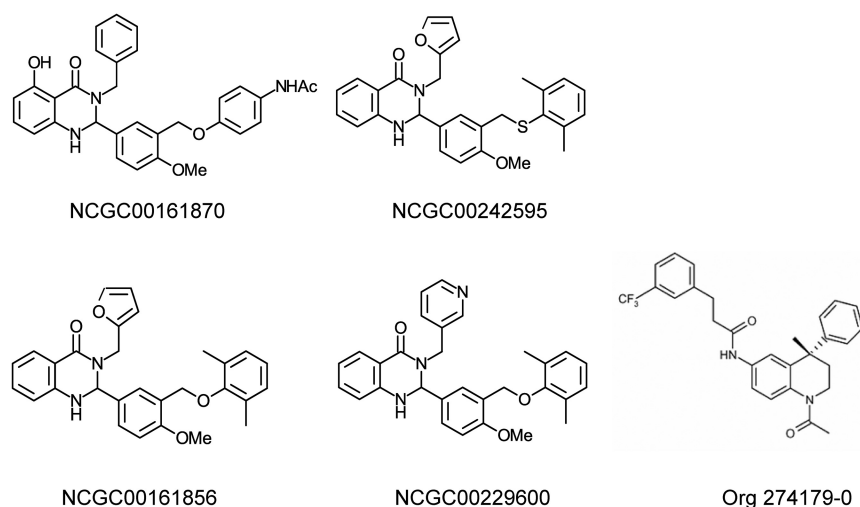
Although we reported on several TSHR agonists earlier (18, 20, 21), the first breakthrough was our report in 2009 describing a TSHR agonist (NCGC00161870) (Fig. 3) that exhibited high affinity, potency, and efficacy at TSHR in a model cell system *in vitro*, in primary cultures of normal thyrocytes *in vitro*, and in mice *in vivo* (22). Moreover, NCGC00161870 was active in mice after oral administration. NCGC00161870 is highly selective and does not activate the two closely related glycoprotein hormone receptors, LH receptor or FSH receptor. As stated above, NCGC00161870 binds to the transmembrane domain of TSHR, in contrast to TSH that binds to the TSHR ectodomain, and is therefore termed an allosteric ligand; the TSH binding site is termed the orthosteric site. To our knowledge, NCGC00161870 is the only effective SML TSHR agonist reported to date.

We have used NCGC00161870 to probe the domains within TSHR that are involved in receptor activation, *i.e.* the structure-activity relationship of a SML agonist with TSHR (23, 24). Specifically, we found that a high propor-

tion of amino acid residues that comprise the binding pocket for NCGC00161870 within the transmembrane domain when mutated lead to constitutively active TSHRs. These findings identify a signaling-sensitive domain within TSHR that may have general applicability to other members of the GPCR family. Moreover, these findings may allow for the design of better TSHR agonists.

NCGC00161870 has been used to better understand the pathophysiology of an unusual form of hypothyroidism caused by TSHR mutations (25). These patients present with subclinical hypothyroidism, *i.e.* with normal circulating levels of thyroid hormones maintained by high levels of TSH, because they are usually heterozygotes with one normal TSHR allele and a mutant TSHR allele. Before our studies, it was postulated that the mutant TSHRs either had a mutation that makes the receptor unable to bind TSH with high affinity or a mutation that makes the mutant receptor incapable of signaling. In both cases, the patients would present with TSH-insensitive (TSH-resistant) hypothyroidism. We studied several mutant TSHRs identified as the cause of this type of hypothyroidism and found that NCGC00161870 could activate the two mutant receptors with mutations in the TSHR ectodomain but could not activate signaling from a receptor with a mutation in the transmembrane domain. We were thus able to demonstrate that some mutations (in the TSHR ectodomain) affected TSH binding but not signaling and others affected TSHR signaling. We suggested that patients with TSH-binding defective mutant receptors could be treated with a SML agonist but that this was not a practical therapeutic approach because these patients were readily treated with thyroid hormone replacement.

Currently, NCGC00161870 and several of its analogs are being further evaluated in preclinical studies for possible submission of an Investigational New Drug application to the Food and Drug Administration to allow testing in human subjects.



**FIG. 3.** Structures of TSHR SMLs. TSHR agonist: NCGC00161870, N-(4-(5-(3-benzyl-5-hydroxy-4-oxo-1,2,3,4-tetrahydroquinazolin-2-yl)-2-methoxybenzyloxy)-phenyl)acetamide. TSHR neutral antagonist: NCGC00242595, 2-(3-((2,6-dimethylphenylthio)methyl)-4-methoxyphenyl)-3-(furan-2-ylmethyl)-2,3-dihydroquinazolin-4(1H)-one. TSHR inverse agonists: NCGC00161856, 2-(3-((2,6-dimethylphenoxy)methyl)-4-methoxyphenyl)-3-(furan-2-ylmethyl)-2,3-dihydroquinazolin-4(1H)-one; NCGC00229600, 2-(3-((2,6-dimethylphenoxy)methyl)-4-methoxyphenyl)-3-(pyridin-3-ylmethyl)-2,3-dihydroquinazolin-4(1H)-one; and Org 274179-0, (S)-N-(1-acetyl-4-methyl-4-phenyl-1,2,3,4-tetrahydro-quinolin-6-yl)-3-(3-trifluoromethylphenyl)-propionamide.

## SML TSHR Antagonists

Although putative TSHR SML antagonists were reported earlier (see below), we reported the first well-characterized SML neutral antagonist (NIDDK-CEB-52) that selectively antagonizes TSHR in 2008 (19). NIDDK-CEB-52 was shown to inhibit activation of TSHR by TSH and TSABs in a model cell system and inhibited up-regulation of thy-

roperoxidase expression by these agonists in primary cultures of human thyrocytes. We suggested that these findings represented proof of principle that SML antagonists could serve as drugs to treat patients with GD.

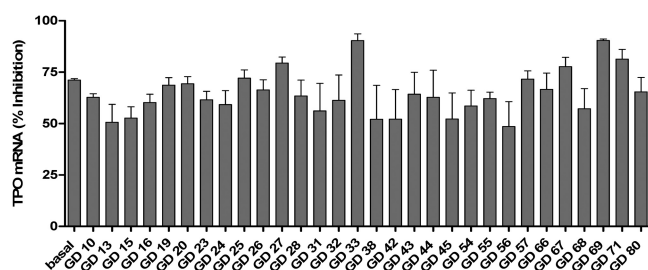
In 2007, a report appeared claiming that the insecticide 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) and Aroclor 1254 (a complex mixture of polychlorinated biphenyls) were inverse agonists at TSHR by showing that these compounds inhibited basal and TSH stimulation of cAMP production in a model cell system (26). In a follow-up study (27), these authors identified a number of structurally similar compounds and found that many of them inhibited TSH stimulation also. Moreover, these compounds were able to inhibit stimulation by LH of its receptor. The authors concluded that there may be an allosteric site on TSHR and LH receptor that mediates inhibition of receptor activation. However, in the original study, these authors found that DDT and Aroclor 1254 inhibited forskolin-stimulated cAMP accumulation also. In our opinion, because forskolin acts downstream of TSHR by activating adenylyl cyclase, the enzyme that synthesizes cAMP, one cannot conclude that these compounds are acting at TSHR.

During the last 2 yr, reports on different compounds discovered by two groups have added important findings to the development of TSHR antagonists. Two reports were published using a compound developed at Merck Sharp & Dohme Corporation (Org 274179-0) (28, 29). In the first report (28), the authors showed that Org 274179-0 (Fig. 2) was a TSHR inverse agonist that inhibited the basal, TSH-stimulated, and TSAb-stimulated signaling in a model system expressing TSHRs, basal signaling by constitutively active mutant TSHRs in a model system, and TSH- and TSAb-stimulated signaling in a rat thyroid cell line (FRTL-5). We had previously described two TSHR inverse agonists (see below). Org 274179-0 has

the advantage of higher potency than the other reported TSHR antagonists (see below). However, it has a significant disadvantage in that it is a partial agonist at the LH receptor. In a follow-up report (29), the authors showed that Org 274179-0 inhibited TSH-, TSAb-, and M22- [a monoclonal antibody generated from the serum of a patient with GD (30)] stimulated cAMP production in orbital fibroblasts derived from patients with GO that had been made to differentiate into adipocytes *in vitro*. This is an important finding (see below) because TSHRs on Graves' orbital fibroblasts are thought to be a primary target for TSABs in the pathogenesis of GO (6).

We have developed a series of TSHR antagonists by chemical modification of the scaffold of our agonist NCGC00161870 (22). Interestingly, all except one analog of the agonist NCGC00161870 were inverse agonists. Only NCGC00242595 (Fig. 3) was a neutral antagonist at TSHR (31). A neutral antagonist is a good tool to be able to distinguish between effects of TSH- or TSAb-stimulated and basal TSHR activation, and therefore it is useful as a probe of TSHR function in addition to its clinical potential. The first report describing this series of antagonists showed that NCGC00161856 (Fig. 3) was the first inverse agonist at TSHR to be described. NCGC00161856, like NCGC00161870, bound to TSHR in a transmembrane pocket and therefore is an allosteric ligand. NCGC00161856 inhibited basal and TSH-stimulated signaling by TSHR and basal signaling by constitutively active mutant TSHRs in a model cell system and lowered the basal levels of expression of four thyroid-specific genes—thyroglobulin, thyroperoxidase, sodium iodide symporter, and TSHR—in primary cultures of normal human thyrocytes. These data were proof of principle that a SML could act as an inverse agonist in normal human thyroid cells.

We developed a better TSHR inverse agonist by modifying NCGC00161856. The new ligand NCGC00229600 (Fig. 3) bound to the allosteric site within the transmembrane domain of TSHR also and inhibited basal and TSH-stimulated signaling (32). Because it would be of greater importance to show that an antagonist would inhibit activation of TSHR signaling stimulated by TSABs from multiple patients with GD, we determined the effect of NCGC00229600 on signaling stimulated by sera from 30 patients with GD. NCGC00229600 inhibited stimulation of signaling by all 30 sera tested both in a model cell system and in primary human thyrocytes in culture. Perhaps more importantly, NCGC00229600 inhibited the up-regulation of thyroperoxidase caused by these TSABs in human thyrocytes (Fig. 4). Thus, we suggested that an antagonist like NCGC00229600 would likely be an effective inhibitor of TSABs from most, if not all, patients with GD and could be used to treat the hyperthyroidism of GD and perhaps GO (see below).



**FIG. 4.** Inhibition of basal and TSAb-induced up-regulation of the expression of thyroperoxidase mRNA in primary cultures of human thyrocytes. NCGC00229600 inhibited the levels by  $65 \pm 2.0\%$  (mean  $\pm$  SEM). Importantly, although the thyrocytes were exposed to NCGC00229600 for 48 h, there was no evidence of toxicity. [Reproduced from S. Neumann *et al.*: A new small-molecule antagonist inhibits Graves' disease antibody activation of the TSH receptor. *J Clin Endocrinol Metab* 96:548–554, 2011 (36), with permission. © The Endocrine Society.]



As stated above, TSHRs on orbital fibroblasts or adipocytes, or both, are thought to be a primary target for TSABs in the pathogenesis of GO (6). It is thought that activation of TSHR by TSABs leads to proliferation of preadipocyte fibroblasts or adipocytes, stimulation of extracellular matrix production, and perhaps differentiation of preadipocytes to adipocytes. Therefore, showing that TSHR antagonists inhibit activation of TSHRs on Graves' orbital fibroblasts (GOFs) would be a step in the development of a medical therapy for GO; there is not a good alternative to surgical decompression at the present time. We determined the effects of NCGC00229600 on undifferentiated GOFs and GOFs differentiated into adipocytes; the adipocytes displayed many lipid vesicles and high levels of the adipocyte-specific gene adiponectin (33). GOFs exhibited higher absolute levels of basal and forskolin-stimulated cAMP production than differentiated adipocytes. Consistent with previous findings, TSH stimulated cAMP production in the majority of differentiated adipocyte strains and less consistently in GOFs. Most importantly, NCGC00229600 reduced both TSH- and M22-stimulated signaling in both cell types. Moreover, NCGC00229600 was a selective TSHR antagonist in these orbital cells because it did not inhibit signaling by activation of prostaglandin D2 receptors, which use the same cAMP signaling pathway as TSHRs. We concluded, as did the authors using Org 274179-0 (29), that SML TSHR antagonists may have a role in the treatment of GO.

## Conclusions

During the last several years, significant advances have been made in the development of SMLs for TSHR. These compounds can be used as probes to better delineate the extrathyroidal actions of TSHR and as lead compounds to develop drugs to treat several thyroid diseases. As described in this Update, some of these agonists, neutral antagonists, and inverse agonists are already being used as probes of TSHR function. And the agonist we have developed is undergoing preclinical studies to determine whether it could be entered into clinical trials for patients with thyroid cancer.

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## References

1. Bassett JH, Williams GR 2008 Critical role of the hypothalamic-pituitary-thyroid axis in bone. *Bone* 43:418–426
2. Blair HC, Robinson LJ, Sun L, Isles C, Davies TF, Zaidi M 2011 Skeletal receptors for steroid-family regulating glycoprotein hormones: a multilevel, integrated physiological control system. *Ann NY Acad Sci* 1240:26–31
3. Bassett JH, Williams AJ, Murphy E, Boyde A, Howell PG, Swinhoe R, Archanco M, Flamant F, Samarut J, Costagliola S, Vassart G, Weiss RE, Refetoff S, Williams GR 2008 A lack of thyroid hormones rather than excess thyrotropin causes abnormal skeletal development in hypothyroidism. *Mol Endocrinol* 22:501–512
4. Davies TF, Ando T, Lin RY, Tomer Y, Latif R 2005 Thyrotropin receptor-associated diseases: from adenomata to Graves disease. *J Clin Invest* 115:1972–1983
5. Rapoport B, McLachlan SM 2007 The thyrotropin receptor in Graves' disease. *Thyroid* 17:911–922
6. Bahn RS 2010 Graves' ophthalmopathy. *N Engl J Med* 362:726–738
7. Duprez L, Parma J, Van Sande J, Rodien P, Dumont JE, Vassart G, Abramowicz M 1998 TSH receptor mutations and thyroid disease. *Trends Endocrinol Metab* 9:133–140
8. Refetoff S 2003 Resistance to thyrotropin. *J Endocrinol Invest* 26:770–779
9. Almandoz JP, Gharib H 2012 Hypothyroidism: etiology, diagnosis, and management. *Med Clin North Am* 96:203–221
10. Franco AT, Malaguarnera R, Refetoff S, Liao XH, Lundsmith E, Kimura S, Pritchard C, Marais R, Davies TF, Weinstein LS, Chen M, Rosen N, Ghossein R, Knauf JA, Fagin JA 2011 Thyrotrophin receptor signaling dependence of Braf-induced thyroid tumor initiation in mice. *Proc Natl Acad Sci USA* 108:1615–1620
11. Haymart MR, Repplinger DJ, Levenson GE, Elson DF, Sippel RS, Jaume JC, Chen H 2008 Higher serum thyroid stimulating hormone level in thyroid nodule patients is associated with greater risks of differentiated thyroid cancer and advanced tumor stage. *J Clin Endocrinol Metab* 93:809–814
12. Pacini F, Castagna MG 2008 Diagnostic and therapeutic use of recombinant human TSH (rhTSH) in differentiated thyroid cancer. *Best Pract Res Clin Endocrinol Metab* 22:1009–1021
13. Duntas LH, Cooper DS 2008 Review on the occasion of a decade of recombinant human TSH: prospects and novel uses. *Thyroid* 18:509–516
14. Woodmansee WW, Haugen BR 2004 A review of the potential uses for recombinant human TSH in patients with thyroid cancer and nodular goiter. *Clin Endocrinol (Oxf)* 61:163–173
15. Fast S, Nielsen VE, Grupe P, Boel-Jørgensen H, Bastholt L, Andersen PB, Bonnema SJ, Hegedüs L 2012 Prestimulation with recombinant human thyrotropin (rhTSH) improves the long-term outcome of radioiodine therapy for multinodular nontoxic goiter. *J Clin Endocrinol Metab* 97:2653–2660
16. Sanders J, Miguel RN, Furmaniak J, Rees Smith B 2010 TSH receptor monoclonal antibodies with agonist, antagonist, and inverse agonist activities. *Methods Enzymol* 485:393–420
17. Vassart G, Pardo L, Costagliola S 2004 A molecular dissection of the glycoprotein hormone receptors. *Trends Biochem Sci* 29:119–126
18. Jäschke H, Neumann S, Moore S, Thomas CJ, Colson AO, Costanzi S, Kleinau G, Jiang JK, Paschke R, Raaka BM, Krause G, Gershengorn MC 2006 A low molecular weight agonist signals by binding to the transmembrane domain of thyroid-stimulating hormone re-

- ceptor (TSHR) and luteinizing hormone/chorionic gonadotropin receptor (LHCGR). *J Biol Chem* 281:9841–9844
19. Neumann S, Kleinau G, Costanzi S, Moore S, Jiang JK, Raaka BM, Thomas CJ, Krause G, Gershengorn MC 2008 A low-molecular-weight antagonist for the human thyrotropin receptor with therapeutic potential for hyperthyroidism. *Endocrinology* 149:5945–5950
  20. Moore S, Jaeschke H, Kleinau G, Neumann S, Costanzi S, Jiang JK, Childress J, Raaka BM, Colson A, Paschke R, Krause G, Thomas CJ, Gershengorn MC 2006 Evaluation of small-molecule modulators of the luteinizing hormone/choriogonadotropin and thyroid stimulating hormone receptors: structure-activity relationships and selective binding patterns. *J Med Chem* 49:3888–3896
  21. Titus S, Neumann S, Zheng W, Southall N, Michael S, Klumpp C, Yasgar A, Shinn P, Thomas CJ, Inglese J, Gershengorn MC, Austin CP 2008 Quantitative high-throughput screening using a live-cell cAMP assay identifies small-molecule agonists of the TSH receptor. *J Biomol Screen* 13:120–127
  22. Neumann S, Huang W, Titus S, Krause G, Kleinau G, Alberobello AT, Zheng W, Southall NT, Inglese J, Austin CP, Celi FS, Gavrilova O, Thomas CJ, Raaka BM, Gershengorn MC 2009 Small molecule agonists for the thyrotropin receptor stimulate thyroid function in human thyrocytes and mice. *Proc Natl Acad Sci USA* 106:12471–12476
  23. Kleinau G, Haas AK, Neumann S, Worth CL, Hoyer I, Furkert J, Rutz C, Gershengorn MC, Schüle R, Krause G 2010 Signaling-sensitive amino acids surround the allosteric ligand binding site of the thyrotropin receptor. *FASEB J* 24:2347–2354
  24. Haas AK, Kleinau G, Hoyer I, Neumann S, Furkert J, Rutz C, Schüle R, Gershengorn MC, Krause G 2011 Mutations that silence constitutive signaling activity in the allosteric ligand-binding site of the thyrotropin receptor. *Cell Mol Life Sci* 68:159–167
  25. Allen MD, Neumann S, Gershengorn MC 2011 Small-molecule thyrotropin receptor agonist activates naturally occurring thyrotropin-insensitive mutants and reveals their distinct cyclic adenosine monophosphate signal persistence. *Thyroid* 21:907–912
  26. Rossi M, Dimida A, Dell'anno MT, Trincavelli ML, Agretti P, Giorgi F, Corsini GU, Pinchera A, Vitti P, Tonacchera M, Maggio R 2007 The thyroid disruptor 1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane appears to be an uncompetitive inverse agonist for the thyrotropin receptor. *J Pharmacol Exp Ther* 320:465–474
  27. Rossi M, Dimida A, Ferrarini E, Silvano E, De Marco G, Agretti P, Aloisi G, Simoncini T, Di Bari L, Tonacchera M, Giorgi F, Maggio R 2009 Presence of a putative steroidal allosteric site on glycoprotein hormone receptors. *Eur J Pharmacol* 623:155–159
  28. van Koppen CJ, de Gooyer ME, Karstens WJ, Plate R, Conti PG, van Achterberg TA, van Amstel MG, Brands JH, Wat J, Berg RJ, Lane JR, Miltenburg AM, Timmers CM 2012 Mechanism of action of a nanomolar potent, allosteric antagonist of the thyroid-stimulating hormone receptor. *Br J Pharmacol* 165:2314–2324
  29. van Zeijl CJ, van Koppen CJ, Surovtseva OV, de Gooyer ME, Plate R, Conti P, Karstens WJ, Timmers M, Saeed P, Wiersinga WM, Miltenburg AM, Fliers E, Boelen A 2012 Complete inhibition of rhTSH-, Graves' disease IgG-, and M22-induced cAMP production in differentiated orbital fibroblasts by a low-molecular-weight TSHR antagonist. *J Clin Endocrinol Metab* 97:E781–E785
  30. Sanders J, Evans M, Premawardhana LD, Depraetere H, Jeffreys J, Richards T, Furmaniak J, Rees Smith B 2003 Human monoclonal thyroid stimulating autoantibody. *Lancet* 362:126–128
  31. Boutin A, Allen MD, Geras-Raaka E, Huang W, Neumann S, Gershengorn MC 2011 Thyrotropin receptor stimulates internalization-independent persistent phosphoinositide signaling. *Mol Pharmacol* 80:240–246
  32. Neumann S, Eliseeva E, McCoy JG, Napolitano G, Giuliani C, Monaco F, Huang W, Gershengorn MC 2011 A new small-molecule antagonist inhibits Graves' disease antibody activation of the TSH receptor. *J Clin Endocrinol Metab* 96:548–554
  33. Neumann S, Pope A, Geras-Raaka E, Raaka BM, Bahn RS, Gershengorn MC 2012 A drug-like antagonist inhibits thyrotropin receptor-mediated stimulation of cAMP production in Graves' orbital fibroblasts. *Thyroid* 22:839–843