Molecular sampling of the allosteric binding pocket of the TSH receptor provides discriminative pharmacophores for antagonist and agonists

Inna Hoyer, Ann-Karin Haas, Annika Kreuchwig, Ralf Schülein and Gerd Krause

Leibniz-Institut für Molekulare Pharmakologie, 13125 Berlin, Germany

Abstract
The TSHR (thyrotropin receptor) is activated endogenously by the large hormone thyrotropin and activated pathologically by auto-antibodies. Both activate and bind at the extracellular domain. Recently, SMLs (small-molecule ligands) have been identified, which bind in an allosteric binding pocket within the transmembrane domain. Modelling driven site-directed mutagenesis of amino acids lining this pocket led to the delineation of activation and inactivation sensitive residues. Modified residues showing CAMs (constitutively activating mutations) indicate signalling-sensitive positions and mark potential trigger points for agonists. Silencing mutations lead to an impairment of basal activity and mark contact points for antagonists. Mapping these residues on to a structural model of TSHR indicates locations where an SML may switch the receptor to an inactive or active conformation. In the present article, we report the effects of SMLs on these signalling-sensitive amino acids at the TSHR. Surprisingly, the antagonistic effect of SML compound 52 was reversed to an agonistic effect, when tested at the CAM Y667A. Switching agonism to antagonism and the reverse by changing either SMLs or residues covering the binding pocket provides detailed knowledge about discriminative pharmacophores. It prepares the basis for rational optimization of new high-affinity antagonists to interfere with the pathogenic activation of the TSHR.

Introduction
GPCRs (G-protein-coupled receptors) are major pharmacological targets for therapeutic applications [1]. Together with FSHR (follicle-stimulating hormone receptor) and LHCG (luteinizing hormone/choriogonadotropin receptor), TSHR (thyroid-stimulating hormone receptor) is a member of the GPHRs (glycoprotein hormone receptors) within family A of GPCRs. TSHR and TSH are involved in a variety of physiological functions in the human body. Stimulation of the receptor leads to increased production and secretion of thyroid hormones, triiodothyronine and thyroxine. The thyroid hormones are primarily responsible for regulation of metabolism such as cell growth and proliferation [2] and are involved in embryonic and early postnatal brain development [3]. Beside its function in the thyroid gland, TSHR is expressed in multiple extrathyroidal tissues (bone, brain, kidney, adipocytes and immune system cells) [4–6]. However, the physiological role in these tissues is poorly known until now.

TSHR is activated endogenously by the large hormone thyrotropin and activated pathologically by auto-antibodies. Such TSHR-stimulating antibodies induce constitutive activation of the receptor and cause overproduction of thyroid hormones leading to hyperthyroidism and GD (Graves’ disease) [7]. One of the prominent symptoms is GO (Graves’ ophthalmopathy). Current treatment options for Graves’ hyperthyroidism and GO are inadequate because they are often invasive and generally target the signs and symptoms of the disease rather than the pathophysiology [8]. A potential approach could be based on the suppression of pathogenic autoantibody activation directly on the TSHR by interference by drug-like SMLs (small-molecule ligands). In contrast with the activating antibodies and thyrotropin that bind to the TSHR extracellular region, synthetic SMLs bind within the transmembrane domain into an additional allosteric binding pocket. Recently, SMLs have been identified which bind in this allosteric binding pocket (reviewed in [9]). The design and application of a HTS (high-throughput screening) assay, especially designed for GPCRs to allow for quick and cost-effective testing of large compound libraries has been developed to identify such SMLs as novel drug candidates. However, the counterpart residues of these SMLs on their targets are studied only incompletely. Using site-directed mutagenesis the amino acids that are covering this allosteric binding pocket of TSHR were recently characterized [10,11].

First, the present review focuses on the recent discovery and characterization of two different mutant types (CAMs [constitutively activating mutations] and inactivating mutations) of residues covering the allosteric binding region. These
signalling-sensitive positions may be potential interaction points of allosteric ligands that may lead to inactivation or activation of the TSHR. Secondly, we examine SMLs that bind into this pocket, located within the transmembrane helical bundle.

Analyses of structure–function relationships between chemical structures of SMLs and functional effects caused by mutation are helpful to gain insights into the interaction between SMLs and wt (wild-type) amino acids covering the allosteric binding site of the TSHR. The knowledge about discriminative pharmacophores on the counterparts, on ligand as well as receptor site, facilitates the extraction of specific fingerprints for agonistic or antagonistic features of SMLs for the TSHR. The long-term aim of these studies is to develop high-affinity and selective antagonists that might have the potential to interfere with pathogenic activation of the TSHR as occurs in GD and GO.

### Allosteric binding pocket in GPHRs

Family A GPCRs have been evolving for 570–700 million years (reviewed in [12]) and possess the general structural topology of an extracellular N-terminal region: seven TMHs (transmembrane helices) connected by three ICLs (intracellular loops) and three ECLs (extracellular loops) and a C-terminal tail. They bind diverse ligands such as amines, purines, lipids or peptides between the transmembrane helical bundle. This transmembrane binding pocket region of family A GCPRs is generally known to be sensitive to endogenous ligand binding [13–16], with the exception of GPHRs. The endogenous glycoprotein hormones of GPHRs bind to a large extracellular orthosteric binding site leading to receptor activation by triggering the transmembrane domain [17,18]. This orthosteric binding pocket is not interesting for pharmacology; on the one hand, the bulky hormone cannot be mimicked by drugs, on the other hand, their large extracellular orthosteric site turned out to be undruggable itself, owing to the large and rather flat binding area (reviewed in [19]). Truncations of the large N-terminal domain [20], mutagenesis studies in the transmembrane domain of TSHR [21] and binding studies of SMLs on LHCGR [22] provided evidence that drugs such as SML bind alternatively to an allosteric site located in the transmembrane helical bundle bundle of GPHRs. Thus, although the orthosteric hormone-binding pocket in the TSHR and other GPHRs is located extracellularly, it appears that the binding pocket in the transmembrane region is also retained in these receptors at a comparable location as known for other GPCRs [23].

In previous studies, amino acid residues covering the TSHR-binding pocket have been characterized. Modelling-driven site-directed mutagenesis of amino acids lining this pocket led to the identification of two types of mutations: CAMs and inactivating mutations. CAMs are located in several helices: V421I (TMH1), Y466A (TMH2), T501A (TMH3), I587V (TMH6), M637C (TMH6), M637W (TMH6), S641A (TMH6), Y643F (TMH6), L645V (TMH6) and Y667A (TMH7). The wt amino acid residues showing CAMs are expected to be key players in stabilizing the basal receptor conformation. These mutations indicate signalling sensitive positions and mark potential trigger points for receptor activation by agonists [11].

Of note, the mutation M637W at position 6.48 (Ballesteros and Weinstein numbering) in TMH6 has a significant effect on the interaction of the receptor with the SML agonist. At this position the strongest constitutive activation within the TMHs of TSHR was found. In the majority of family A GPCRs, tryptophan is located at position 6.48 and has been known to be involved in receptor activation. According to these data one can speculate that Met637 is involved in both stabilizing the basal and supporting the active conformation [11]. This indicates that this position is a potential interaction partner for agonists. However, this also implies that antagonists should avoid this activation-sensitive Met637 position.

The identified inactivating mutations, also termed silencing mutations, led to an impairment of basal TSHR activity. The TSHR three-dimensional homology model visualizes the spatial distribution of mutants within the binding pocket. Two spatial clusters of silencing mutations flanking the allosteric binding pocket have been described in [10]. Cluster I is arranged between amino acids Val502 (TMH3), Leu552 (TMH4), Tyr582 (TMH5) and Met572 at ECL2. Silencing mutations in the ECL2 were previously reported [24]. Amino acids Val425 (TMH1), Leu467 (TMH2) and Leu655 (TMH7) form cluster II. Such silencing mutations switch the receptor to a more inactive conformation. Potential antagonistic SMLs should be able to constrain the TSHR in an inactive conformation by interacting with these positions known for their ability to reduce the basal TSHR activity. Taken together, all these signalling-sensitive amino acids residues (extracted from [25] and summarized in Table 1) indicate locations where SMLs may shift the receptor to either an inactive or an active conformation. CAMs mark contact points for agonists and silencing mutations mark the contact points for antagonists.

### Allosteric modulators

The first small molecule interacting with the TSHR was originally developed as an agonist for the LHCGR [26]. Molecular modelling and functional experiments guided the chemical derivation of the LHCGR agonist and led to the identification of the first TSHR-specific partial agonist Org-41841 [27] as well as low-affinity antagonist c52 (compound 52) [20]. Both compounds share the same basic thienopyrimidine scaffold structure. This knowledge, from in silico as well as functional studies, directed the following investigations that resulted in the identification of several different TSHR ligands [23,28]. To gain a better understanding of the interactions of SMLs within the binding pocket we report about the effects of SMLs on signalling-sensitive amino acids at TSHR and use c52 and Org-41841 as examples. The two SMLs are very similar and differ only by an extended side chain on the aromatic ring of the antagonist (see Figure 1 and [27]). Based on our three-dimensional homology...
Table 1 | Residues of the TSH receptor covering the allosteric binding pocket that show signalling-sensitive effects upon mutation either as constitutive activating mutation (CAMs) or as silencing mutation

Mutations known to cause disease-relevant effects are indicated for inactivating pathogenic and for activating pathogenic mutations.

| Location | Ballesteros and Weinstein numbering | Amino acid human TSHR | Silencing mutation | CAM | Reference |
|----------|------------------------------------|-----------------------|-------------------|-----|-----------|
| TMH1     | 1.39 Tyler Val421                   | Ile                   |                   |     | [11]      |
|          | 1.42 Tyler Val424                   | Ile                   |                   |     | [10]      |
| TMH2     | 2.53 Tyler Met463                   | Val†                  |                   |     | [29]      |
|          | 2.56 Tyler Tyr466                   | Ala                   |                   |     | [11]      |
|          | 2.57 Tyler Leu467                   | Pro*, Val             |                   |     | [10,30]   |
|          | 2.64 Tyler Asp474                   | Glu                   |                   |     | [31]      |
| TMH3     | 3.32 Tyler Thr501                   | Ile                   |                   |     | [32]      |
|          | 3.36 Tyler Ser505                   | Asn†, Arg             |                   |     | [33–37]   |
|          | 3.38 Tyler Leu507                   | Ser                   |                   |     | [36]      |
|          | 3.40 Tyler Val509                   | Ala                   |                   |     | [36]      |
| ECL2     | 5.39                               | Leu                   | Ala, Phe, Val, Thr† |     | [24,38]   |
|          |                                    | Leu                   | Ala, Phe          |     | [21,24]   |
|          |                                    | Met572                | Ala               |     | [24,39]   |
| TMH5     | 5.43                               | Val586                | Ile                |     | [10]      |
|          | 5.44                               | Leu587                | Val                |     | [11]      |
|          | 5.51                               | Phe594                | Ile                |     | [10]      |
| TMH6     | 6.45                               | Phe634                | Ile                |     | [10]      |
|          | 6.48                               | Met637                | Cys, Trp           |     | [11]      |
|          | 6.50                               | Pro639                | Ser†               |     | [40,41]   |
|          | 6.51                               | Ile640                | Leu                | Lys†, Val | [24,42]   |
|          | 6.52                               | Ser641                | Leu                | Alα   | [11]      |
|          | 6.53                               | Phe642                | Ile                |     | [10]      |
|          | 6.54                               | Tyr643                | Ala                | Phe   | [10,11]   |
|          | 6.56                               | Leu645                | Val                |     | [11]      |
| TMH7     | 7.40                               | Leu665                | Val                |     | [10]      |
|          | 7.42                               | Tyr667                | Ala                |     | [11]      |

The model of the TSHR, the localization of both compounds within the binding pocket in the transmembrane domain was predicted and shown to be very similar, but differs in a shifted orientation of the molecules.

The partial agonist Org-41841 is smaller and binds deeper into the binding region and is therefore able to interact with the Met637 at position 6.48 on TMH6. This is consistent with the finding that position Met637 has been identified as a key player of activation where the strongest CAM was found [11]. In contrast, c52 revealed a slightly shifted localization towards the extracellular side in the binding pocket in comparison to the partial agonist Org-41841. Thus c52 cannot reach Met637. The shifted orientation of c52 within the binding pocket is the consequence of an enlarged substituent on the phenyl group of this compound. Moreover, this prolonged side chain extends into the region where more inactivating mutations were found. Docking studies predicted a sensitive amino acids residue Tyr667 at TMH7 (position 7.42) as a contact point for c52. In the molecular model, c52 is constrained by the Tyr667 residue, therefore c52 is not able to reach Met637.

Effect of allosteric modulators

Surprisingly, the antagonistic effect of SML c52 was reversed to an agonistic effect, when tested at the constitutively activating TSHR mutant Y667A. The side chain reduction by an alanine mutation releases c52 that then subsequently hits Met637, which is located at the bottom of the binding pocket at position 6.48, where a highly conserved tryptophan at TMH6 exists on other GPCRs. Met637 was identified as a key residue for activation by showing a very strong constitutive activation that indicates its potential as an interaction partner for agonists. These reverse effects confirm not only the predicted binding site for c52, but also provide details about distinguishing locations for antagonists and agonist.

Docking studies of partial agonist Org-41841 into the three-dimensional TSHR homology model predicted a hydrogen bond interaction between the amino group of the ligand and residue Glu506 (TMH3). Interestingly, mutant E506A did not lead to receptor activation by stimulation with Org-41841, whereas the activation by TSH is not affected. The alanine substitution at this highly conserved position

© 2011 The Author(s) The author(s) has paid for this article to be freely available under the terms of the Creative Commons Attribution Non-Commercial Licence (http://creativecommons.org/licenses/by-nc/2.5/) which permits unrestricted non-commercial use, distribution and reproduction in any medium, provided the original work is properly cited.
Glu506 resulted in a breakdown of receptor activation. These data indicates ligand–receptor interaction and supports the predicted orientation of the ligand within the pocket [21].

In conclusion, elucidation of detailed activating and inactivating patterns among residues covering the allosteric binding site of TSHR allows for directed manipulations to switch from agonism to antagonism and vice versa. Modifications either at signalling-sensitive receptor positions at the allosteric binding pocket or at an SML can modify or even reverse the activation state of TSHR. This strategy revealed detailed knowledge about discriminative pharmacophores for agonistic and antagonistic features of SMLs, which can now be used to guide future studies. The information gained about complementary properties of the allosteric binding region and the small molecule ligands are the basis for the optimization of high-affinity antagonists for the TSHR for treatment of GO or constitutive activation by pathogenic mutations.

**Funding**

This work was funded by the Deutsche Forschungsgemeinschaft [grant number KR 1273/4-1].

**References**

1. Schöneberg, T., Schulz, A., Biebermann, H., Hermosdorf, T., Rompler, H. and Sangkuhl, K. (2004) Mutant G-protein-coupled receptors as a cause of human diseases. Pharmacol. Ther. 104, 173–206
2. Vassart, G. and Droumont, J.E. (1992) The thyrotropin receptor and the regulation of thyrotropin function and growth. Endocr. Rev. 13, 596–611
3. Zoeller, R.T. and Rovet, J. (2004) Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings. J. Neuroendocrinol. 16, 809–818
4. Bahn, R.S., Dutton, C.M., Natt, N., Jowa, W., Spitzweg, C. and Heufelder, A.E. (1998) Thyrotropin receptor expression in Graves’ orbital adipose/connective tissues: potential autoantigen in Graves’ ophthalmopathy. J. Clin. Endocrinol. Metab. 83, 998–1002
5. Bassett, J.H. and Williams, G.R. (2008) Critical role of the hypothalamic–pituitary–thyroid axis in bone. Bone 43, 418–426
6. Davies, T., Marans, R. and Latif, R. (2002) The TSH receptor reveals itself. J. Clin. Invest. 110, 161–164
7. Ragoppo, B. and McIachlan, S.M. (2007) The thyrotropin receptor in Graves’ disease. Thyroid 17, 911–922
8. Bahn, R.S. (2012) Autoimmunity and Graves’ disease. Clin. Pharmacol. Ther. 91, 577–579
9. Gershengorn, M.C. and Neumann, S. (2012) Update in TSH receptor agonists and antagonists. J. Clin. Endocrinol. Metab. 97, 4287–4292
10. Haas, A.K., Kleinau, G., Hoyer, I., Neumann, S., Forkert, J., Rutz, C., Schulein, R., Gershengorn, M.C. and 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
11. Kleinau, G., Haas, A.K., Neumann, S., Worth, C.L., Hoyer, I., Forkert, J., Rutz, C., Gershengorn, M.C., Schulein, R. and Krause, G. (2010) Signaling-sensitive amino acids surround the allosteric ligand binding site of the thyrotropin receptor. FASEB J. 24, 2347–2354
12. Strötmann, R., Schock, K., Boselt, I., Staubert, C., Riss, A. and Schöneberg, T. (2011) Evolution of GPCR: change and continuity. Mol. Cell. Endocrinol. 331, 170–178
13. Gershengorn, M.C. and Osman, R. (2001) Minireview: insights into G protein-coupled receptor function using molecular models. Endocrinology 142, 2–10
14. Gliomin, D.E., Foord, S.M., Blaney, F.E. and Garland, S.L. (2009) Definition of the G protein-coupled receptor transmembrane bundle binding pocket and calculation of receptor similarities for drug design. J. Med. Chem. 52, 4429–4442
15. Stenkamp, R.E., Teller, D.C. and Palczewski, K. (2005) Rhodopsin: a structural primer for G-protein coupled receptors. Arch. Pharm. 338, 209–216
16. Tunaru, S., Lattig, J., Kero, J., Krause, G. and Offermanns, S. (2005) Characterization of determinants of ligand binding to the nicotinic acid receptor GP109A (HM74A/PUMA-G). Mol. Pharmacol. 68, 1271–1280
17. Kleinau, G., Brehm, M., Wiedermann, U., Labudde, D., Leser, U. and Krause, G. (2007) Implications for molecular mechanisms of glycoprotein hormone receptors using a new sequence-structure-function analysis resource. Mol. Endocrinol. 21, 574–580
G-Protein-Coupled Receptors: from Structural Insights to Functional Mechanisms

217

18 Kleinau, G. and Krause, G. (2009) Thyrotropin and homologous glycoprotein hormone receptors: structural and functional aspects of extracellular signaling mechanisms. Endocrinol. Rev. 30, 133–151

19 Nagayama, Y. and Rapoport, B. (1992) The thyrotropin receptor 25 years after its isolation: new light after its molecular cloning. Mol. Endocrinol. 6, 145–156

20 Neumann, S., Kleinau, G., Costanzi, S., Moore, S., Jiang, J.K., Raaka, B.M., Thomas, C.J., Krause, G. and Gershengorn, M.C. (2008) A low-molecular-weight antagonist for the human thyrotropin receptor with therapeutic potential for hyperthyroidism. Endocrinology 149, 5945–5950

21 Jaschke, H., Neumann, S., Moore, S., Thomas, C.J., Colson, A.O., Costanzi, S., Kleinau, G., Jiang, J.K., Paschke, R., Raaka, B.M. et al. (2006) A low molecular weight agonist signals by binding to the transmembrane domain of thyroid-stimulating hormone receptor (TSHR) and luteinizing hormone/choriogonadotropin receptor (LCGR). J. Biol. Chem. 281, 9841–9848

22 Heitman, L.H., Kleinau, G., Brussee, J., Krause, G. and Ijzerman, A.P. (2012) Determination of different putative allosteric binding pockets at the lutropin receptor by using diverse drug-like low molecular weight ligands. Mol. Cell. Endocrinol. 351, 326–336

23 Neumann, S., Haung, W., Titus, S., Krause, G., Kleinau, G., Alberobello, A.T., Zheng, W., Southall, N.T., Inglese, J., Austin, C.P. et al. (2009) Small-molecule agonists for the thyrotropin receptor stimulate thyroid function in human thyocytes and mice. Proc. Natl. Acad. Sci. U.S.A. 106, 12471–12476

24 Kleinau, G., Claus, M., Jaeschke, H., Mueller, S., Neumann, S., Paschke, R. and Krause, G. (2007) Contacts between extracellular loop two and transmembrane helix six determine basal activity of the thyrotropin-stimulating hormone receptor. J. Biol. Chem. 282, 518–525

25 Kreuchwig, A., Kleinau, G., Kreuchwig, F., Worth, C.L. and Krause, G. (2011) Research resource: update and extension of a glycoprotein hormone receptors web application. Mol. Endocrinol. 25, 707–712

26 van Straten, N.C., Schoonus-Gerritsma, G.G., van Someren, R.G., Draaijer, J., Adang, A.E., Timmers, C.M., Hansen, R.G. and van Boeckel, C.A. (2002) The first orally active low molecular weight agonists for the LH receptor: thiophenopyr(im)idines with therapeutic potential for ovulation induction. ChemBioChem. 3, 1023–1026

27 Moore, S., Jaeschke, H., Kleinau, G., Neumann, S., Costanzi, S., Jiang, J.K., Childress, J., Raaka, B.M., Colson, A., Paschke, R. et al. (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

28 van Kopen, C.J., de Goeijer, M.E., Karstens, W.J., Plat, R., Conti, P.G., van Achterberg, T.A., van Amstel, M.G., Brands, J.H., Wat, J., Berg, R.J. et al. (2012) Mechanism of action of a nanomolar potent, allosteric antagonist of the thyroid-stimulating hormone receptor. Br. J. Pharmacol. 165, 2314–2324

29 Fuhrer, D., Warner, J., Sequeira, M., Paschke, R., Gregory, J. and Ludgate, M. (2000) Novel TSHR germ-line mutations (Met463Val) masquerading as Graves’ disease in a large Welsh kindred with hyperthyroidism. Thyroid 10, 1035–1041

30 Alberti, L., Proverbio, M.C., Costagliola, S., Romoli, R., Boldrighini, B., Vigone, M.C., Weber, G., Chiumello, G., Beck-Pecco, P. and Persani, L. (2002) Germine mutations of TSH receptor gene as cause of nonautoimmune subclinical hypothyroidism. J. Clin. Endocrinol. Metab. 87, 2560–2565

31 Neumann, S., Claus, M. and Paschke, R. (2005) Interactions between the extracellular domain and the extracellular loops as well as the 6th transmembrane domain are necessary for TSH receptor activation. Eur. J. Endocrinol. 152, 625–634

32 Montanelli, L., Van Dume, J.J., Smits, G., Bonomi, M., Rodien, P., Devor, E.J., Moffat-Wilson, K., Pardo, L., Vassart, G. and Costagliola, S. (2004) Modulation of ligand selectivity associated with activation of the transmembrane region of the human follicitropin receptor. Mol. Endocrinol. 18, 2061–2073

33 Holzapfel, H.P., Wonerow, P., von Petykowksi, W., Henschken, M., Scherbaum, W.A. and Paschke, R. (1997) Sporadic congenital hyperthyroidism due to a spontaneous germline mutation in the thyrotropin receptor gene. J. Clin. Endocrinol. Metab. 82, 3879–3884

34 Wonerow, P., Chey, S., Fuhrer, D., Holzapfel, H.P. and Paschke, R. (2000) Functional characterization of a constitutively activating thyrotropin receptor mutations. Clin. Endocrinol. 53, 461–468

35 Glayesery, S., Gavant, C., Lefort, A., Van Sande, J., Costagliola, S., Pardo, L. and Vassart, G. (2002) A conserved Asn in TM7 of the thyrotropin receptor is a common requirement for activation by both mutations and its natural agonist. FEBS Lett. 517, 195–200

36 Kosugi, S., Hsu, N., Okamoto, H., Sagawa, H. and Mori, T. (2000) A novel activating mutation in the thyrotropin receptor gene in an autonomously functioning thyroid nodule developed by a Japanese patient. Eur. J. Endocrinol. 143, 471–477

37 Tonacchera, M., Van Sande, J., Cetani, F., Swillens, S., Schwart, C., Winiszewski, P., Portmann, L., Dumont, J.E., Vassart, G. and Parma, J. (1996) Functional characteristics of three new germline mutations of the thyrotropin receptor gene causing autosomal dominant toxic thyroid hyperplasia. J. Clin. Endocrinol. Metab. 81, 547–554

38 Parma, J., Van Sande, J., Swillens, S., Tonacchera, M., Dumont, J. and Vassart, G. (1995) Somatic mutations causing constitutive activity of the thyrotropin receptor are the major cause of hyperfunctioning thyroid adenomas: identification of additional mutations activating both the cyclic adenosine 3′,5′-monophosphate and inositol phosphate-Ca2+ cascades. Mol. Endocrinol. 9, 725–733

39 Kleinau, G., Jaeschke, H., Mueller, S., Worth, C.L., Paschke, R. and Krause, G. (2008) Molecular and structural effects of inverse agonistic mutations on signaling of the thyrotropin receptor—a basally active GPCR. Cell. Mol. Life Sci. 65, 3664-3676

40 Agretti, P., De Marco, G., Collecchi, P., Chiavoto, L., Vitti, P., Pinchera, A. and Tonacchera, M. (2003) Proper targeting and activity of a nonfunctioning thyroid-stimulating hormone receptor (TSHR) combining an inactivating and activating TSHr mutation in one receptor. Eur. J. Biochem. 270, 3839–3847

41 Tonacchera, M., Chiavoto, L., Pinchera, A., Agretti, P., Fiore, E., Cetani, F., Rocchi, R., Viacava, P., Miccoli, P. and Vitti, P. (1996) Hyperfunctioning thyroid nodules in toxic multinodular goiter share activating thyrotropin receptor mutations with solitary toxic adenoma. J. Clin. Endocrinol. Metab. 83, 492–498

42 Gozu, H.I., Bircan, R., Krohn, K., Muller, S., Vural, S., Gezen, C., Sargin, H., Yavuzer, D., Sargin, M., Cinkoglu, B. et al. (2006) Similar prevalence of somatic TSH receptor and Gsalpha mutations in toxic thyroid nodules in geographical regions with different iodine supply in Turkey. Eur. J. Endocrinol. 155, 535–545

Received 5 November 2012
doi: 10.1042/BST20120319