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The New Pharmacological Approaches for the Regulation of Functional Activity of G Protein-Coupled Receptors

Alexander O. Shpakov and Kira Viktorovna Derkach

Abstract

The G protein-coupled receptors (GPCRs), a large family of the receptors that specifically interact with a number of signal molecules, play a key role in the regulation of fundamental cell processes, and the pharmacological action of over 40% of drugs is carried out through GPCRs. In the last years, a significant progress was made in the creation of selective regulators of GPCRs interacting with their allosteric sites, such as the synthetic peptides corresponding to intracellular regions of receptors (GPCR-peptides) and the low-molecular weight agonists and antagonists of GPCRs. This review describes the recent results obtained by us and other authors in the development of GPCR-peptides and low-molecular weight agonists and the prospects of their use in clinics.

Keywords: low-molecular weight agonist, G protein-coupled receptor, allosteric regulation, GTP-binding protein, thienopyrimidine

1. Introduction

The G protein-coupled receptors (GPCRs) are a large family of cell surface receptors that specifically interact with a number of signal molecules, such as amino acids, nucleotides, peptides, proteins, lipids and odorants. GPCRs play a key role in the regulation of fundamental cell processes including growth, metabolism, differentiation, motility and apoptosis [1, 2]. The evolution of GPCRs has about 700 million years, and they are characterized by the similarity of the structural and functional organization and membrane topology [3]. GPCRs contain seven transmembrane regions (TM) forming a transmembrane channel, N-terminal extracellular and C-terminal intracellular domains (CTD) and three extracellular
and three intracellular loops (ICLs). The ICLs of agonist-bound GPCR interact with αβγ-heterotrimeric GTP-binding proteins (G proteins) and β-arrestins, which regulate activity of adenylyl cyclase (AC), phospholipase C, phosphatidylinositol 3-kinase, mitogen-activated protein kinases and G protein-gated ion channels. In most cases, the membrane-proximal regions of the second and third ICLs are involved in the interaction with G proteins, while the third ICL and CTD with β-arrestins. The extracellular regions are responsible for ligand recognition and participate in the formation of high-affinity ligand-binding (orthosteric) site, which is usually located in the transmembrane channel of GPCRs and, in certain receptors, is placed in a large ectodomain.

The action of over 40% of the currently used drugs affecting physiological and biochemical processes is carried out through GPCRs [4]. The changes in GPCRs and their signaling cascades lead to a large number of diseases, indicating that the search of new selective and effective regulators of GPCRs is the critical challenge to medical endocrinology and biochemistry. Nowadays, one of the most widely used approaches to create GPCR regulators is the development of synthetic analogs of natural hormones, which specifically interact with the orthosteric site of GPCR and having high efficiency and selectivity. This approach began to give very good results when the 3D structure for a number of GPCRs was established using X-ray analysis of their crystal forms [5]. In the last years, a significant progress was made in the development of GPCR regulators interacting with the allosteric sites of receptor. For this purpose, a few strategies can be used, and among them, greatest interests are the design of peptides corresponding to intracellular regions of GPCRs (GPCR-peptides) and the creation of low-molecular weight (LMW) agonists and antagonists. The GPCR-peptides and LMW regulators specifically bind to the allosteric sites of receptor located in the ICLs and in the transmembrane domain, respectively.

This review describes the recent results obtained by us and other authors in the development of GPCR-peptides and LMW agonists and the prospects of their use in clinic.

2. The GPCR-peptides and their lipophilic derivatives

Currently, a lot of data were obtained that synthetic peptides corresponding to intracellular regions of GPCRs specifically influence the activity of cognate receptors and intracellular pathways dependent on them [6–9]. Since the regions interacting with G proteins and β-arrestins are located primarily in membrane-proximal segments of ICLs, conformation of these regions in full-size GPCR is stabilized by hydrophobic segments of TM and by interactions between N- and C-termini of ICLs. Therefore, the modification of GPCR-peptides by hydrophobic radicals and lipophilic amino acid sequences simulating TM, as well as the cyclization of GPCR-peptides should lead to significant increase in biological activity of modified GPCR-peptides, which was confirmed by the in vivo and the in vitro experiments [6, 8, 10, 11]. GPCR-peptides modified by hydrophobic radicals and amino acid sequences, which are similar in physicochemical properties to TM segments, are able to penetrate the plasma membrane, to incorporate into the TM/ICL interfaces formed by membrane-proximal segments of
ICLs and cytoplasmic portion of TM, and to interact with the allosteric sites of cognate GPCR (Figure 1). The GPCR-peptides modified by hydrophobic radicals, primarily by palmitoyl and myristoyl radicals, are designated as pepducins [6]. They influence the signal transduction, acting as intracellular allosteric agonists or antagonists, and are capable of triggering the appropriate cell response in the absence of hormonal stimulus [12–16]. As pepducins specifically interact with complementary regions of cognate GPCR, their effects are receptor specific. Pepducins do not affect even the closely related receptors and are active only in tissues and cells where the receptors homologous to them are expressed [17, 18].

Lipophilic derivatives of GPCR-peptides corresponding to ICLs of types 1, 2 and 4 protease-activated receptors (PARs) influence platelet aggregation, inflammation, angiogenesis, apoptosis, transformation and metastasis [19]. Pepducin P1pal-7 (PZ-128), a derivative of N-terminal segment of the third ICL (ICL3) of PAR1, functions as antagonist and significantly inhibits PAR1-mediated platelet aggregation and arterial thrombosis, being a good alternative to LMW PAR1-antagonists used to treat arterial thrombosis. The compound PZ-128 suppresses the tumor growth and metastasis, reduces the cell viability in breast, ovarian and lung carcinoma cells, inhibits PAR1-mediated migration of lung cancer cells and decreases lung tumor growth [20, 21]. The efficiency of PZ-128 is comparable to that of Bevacizumab, a widely used antitumor agent and angiogenesis inhibitor. Lipophilic derivatives of GPCR-peptides corresponding to ICLs of the chemokine receptors CXCR1, CXCR2 and CXCR4 also possess the potent antitumor activity [12, 13]. The CXCR4-derived pepducins with high efficiency

Figure 1. The molecular mechanisms of action of GPCR-peptides modified by hydrophobic radicals. Specific interaction of GPCR-peptide (in green) with complementary region (in purple) of the cognate receptor leads to: (1) the disconnection of the interaction between receptor region (in green) homologous to GPCR-peptide with complementary region; (2) the changes in conformation of complementary region that is involved in the interaction with G protein α-subunit; and, finally, (3) the activation or, on the contrary, inhibition of G protein α-subunit and downstream effector enzymes.
suppress the survival and metastasis of disseminated lymphoma xenografts, which can be the basis of their application in treating lymphoid malignancies [13]. The pepducin P2pal-18S corresponding to the ICL3 of PAR2, an allosteric antagonist of this receptor, and its analogs protect acinar cells against injury induced by bile acid and reduce the severity of acute biliary pancreatitis in mice, which indicates a good opportunity for their use in management of patients with high risk of acute biliary pancreatitis [22].

We synthesized and studied a large number of lipophilic GPCR-peptides that correspond to the ICL3 of type 1 relaxin receptor (RXFP1), 5-hydroxytryptamine receptor of the type 6 (5-HT_6R) and the receptors of luteinizing hormone/chorionic gonadotropin (LH/ChG-R) and thyroid-stimulating hormone (TSHR). These receptors are involved in the regulation of nervous, endocrine and reproductive systems. Binding of these receptors to hormones induces activation of G_s proteins and the enzyme adenylyl cyclase (AC), which leads to increase in intracellular cyclic adenosine monophosphate (cAMP) level and activation of cAMP-dependent enzymes and transcriptional factors. The choice of the ICL3 for synthesis of GPCR-peptides was due to the fact that this loop is responsible for specific binding and activation of G proteins [23].

The C-palmitoylated 11-mer peptide QVKKE(Nle)ILAKR_619–629K(Pal) corresponding to C-terminal portion of the ICL3 of RXFP1 stimulated AC activity and GTP-binding capacity of G_s proteins and, in addition, reduced regulatory effects of relaxin. The influence of 11-mer palmitoylated peptide on the AC signaling system (ACSS) was more pronounced as compared with 15-mer peptide 615–629 lacking fatty acid radical, while unmodified 11-mer peptide was not active [14]. The C-palmitoylated peptide KHSRKALKASL_258–268K(Pal)A corresponding to the ICL3 of 5-HT_6R stimulated the activity of G_s proteins and AC much more effectively than its unmodified analog [15]. The similar results were obtained for PAR1-derived peptides. Peptide PAR1-295-313, lacking hydrophobic radical, had a little effect on activity of calcium channels, while its palmitoylated analog caused a rapid stimulation of Ca^{2+} influxes [6]. The regulatory effects of peptides 619-629-K(Pal) and 258-268-K(Pal)A were tissue specific. The peptides 619-629-K(Pal) stimulated AC in plasma membranes isolated from the myocardium and brain that are enriched by the receptors RXFP1, but had no effect in the skeletal muscles where there are no RXFP1 [14]. Palmitoylated 5-HT_6R-peptide was effective in the brain rich in 5-HT_6R, but not in the myocardium and testes where 5-HT_6R are absent or expressed very little [15]. These results give grounds to make a conclusion that modification of GPCR-peptides by hydrophobic radicals is one of the most perspective approaches to enhance their specific biological activity.

In the in vitro experiments, the lysine-palmitoylated peptide 612-627-K(Pal)A, the derivatives of C-terminal portion of the ICL3 of TSHR, stimulated the basal AC activity and GTP-binding of G_s proteins in the thyroidal membranes and reduced the AC activity stimulated by TSH [16]. In the in vivo experiments, single and 3-day treatment of rats with intranasally or intramuscularly administered peptide 612-627-K(Pal)A led to increase of thyroid hormones level and, in the case of 3-day treatment, to decrease of plasma TSH concentration. Two hours after a single intranasal administration of peptide 612-627-K(Pal)A (450 μg/kg), the level of free thyroxine (fT_4) and total 3,5,3′-triiodothyronine (tT_3) was increased by 31 and 37%. The
stimulating effect of 612-627-K(Pal)A on thyroid function was enhanced on the second day of treatment and then weakened, which was due to the decrease of thyroid sensitivity to the peptidic TSHR agonist. The evidence for this was the weakening of stimulating effect of intranasally administered thyrotropin-releasing hormone on the production of thyroid hormones in rats treated for 2 days with 612-627-K(Pal)A [11]. Unmodified peptide 612-627-KA possessed a low activity in the in vitro experiments and had no influence on thyroid status of animals irrespective of the mode of administration [11, 16]. Thus, the ability of peptide 612-627-K(Pal)A to stimulate the production of thyroid hormones gives grounds to consider it as a prototype for creating the novel thyroid regulators.

To estimate the effect of localization and length of fatty acid radicals on biological activity of GPCR-peptides, we synthesized the series of acylated analogs of peptide 562–572 corresponding to the ICL3 of LH/ChG-R and studied their influence on gonadotropin-sensitive ACSS in rat testicular membranes. The lipophilic derivatives of peptide 562–572 containing palmitoyl and decanoyl radicals at the N- or C-terminus, or at both termini, were investigated. We showed that lipophilic peptides modified by fatty acid radicals at the C-terminus, where in full-size LH/ChG-R the sixth TM is located, stimulated in a dose-dependent manner the basal AC activity and GppNHp binding of Gs proteins and reduced the AC stimulating effect of human ChG [24]. The C-palmitoylated peptide 562–572 was much more active than its decanoyl counterpart. The N-acylated peptides had no effect on the ACSS. These data support the view that hydrophobic radical in GPCR-peptides mimics TM and must be comparable with it in the size and hydrophobicity. The establishment of the criteria for modification of GPCR-peptides with hydrophobic radicals is one of the most promising ways to create drugs capable of regulating the biochemical and physiological processes in vivo.

The progress achieved in development of GPCR-peptides open up prospects for their wide application in pharmacology and medicine as drugs to treat cancer, immunological, endocrine, cardiovascular and other diseases, as well as in fundamental biology as an instrument to study the molecular mechanisms of GPCR interaction with ligands and intracellular regulatory and effector proteins. The modification of GPCR-peptides can significantly change their selectivity, efficiency, bioavailability and stability, which allows unlimited expand the field of the use of the peptides. Note, in the last years, the works were carried out on the development of peptides corresponding to functionally active regions of receptor tyrosine kinases, G proteins and the enzymes generating the second messengers [7, 9].

3. The low-molecular weight (LMW) allosteric regulators of receptors of glycoprotein hormones

Unlike most of the other GPCRs, LH/ChG-R and TSHR have a large N-terminal extracellular domain (ectodomain), which forms orthosteric site for high-affinity binding of glycoprotein hormones [25, 26]. Typically, high-affinity binding of ligand occurs in the orthosteric site located in transmembrane channel of receptor. The transmembrane channel of LH/ChG-R and TSHR, on the contrary, involves the allosteric site that remains free when receptor is
occupied by hormone. The binding of glycoprotein hormones causes conformational rearrangements in ectodomain and leads to the changes in the interaction between ectodomain and extracellular loops of GPCR. This induces the conformational changes in the transmembrane channel and allosteric site located therein, in ICLs forming G protein-binding surface of receptor and causes activation of G proteins and intracellular effectors, including AC and phosphoinositide-specific phospholipase C (PLC) [26, 27].

The first LMW ligands of LH/ChG-R, the derivatives of thienopyrimidines, were discovered in 2002 [28]. They possess the ability to penetrate into transmembrane channel, specifically interact with the allosteric site located therein and, as a result, activate LH-dependent signaling cascades. Based on the structure of thienopyrimidine derivatives, in the recent years, the LMW agonists and antagonists of TSHR were developed, which opened a new way in the pharmacological treatment of thyroid diseases [29, 30].

The LH and human ChG, the endogenous ligands of LH/ChG-R, are the αβ-heterodimers consisting of highly conservative α-subunit and variable β-subunit, and the β-subunit determines specificity of gonadotropins binding to LH/ChG-R ectodomain. Meanwhile, the use of LH and human ChG in medicine is limited by the rapid development of resistance of reproductive tissues to these hormones, their immunogenicity, and the requirement of parenteral administration of gonadotropins. All this makes it necessary to develop the new LH/ChG-R regulators, which differ in the mechanisms of action from gonadotropins. Searching for such regulators led to the discovery of several classes of chemical compounds, including the pyrazole and 1,3,5-terphenyl derivatives. The most active among them were the thienopyrimidine derivatives, such as compound Org 41,841, N-tert-butyl-5-amino-4-(3-methoxyphenyl)-2-(methylthio)thieno[2,3-d]pyrimidine-6-carboxamide and its analog Org 43553 [28, 31].

The Org 43553 binds specifically to LH/ChG-R (K_d, 2.4 nM), and the treatment of cells with [125I]-ChG does not cause the dissociation of Org 43553. At nanomolar concentrations, Org 43553 activates AC and cAMP-dependent transcriptional factors with the efficiency of 62 and 80% of that LH, but had a little effect on the activity of receptor of follicle-stimulating hormone and TSHR [31]. It is known that gonadotropins via G_q protein stimulate PLC, and activation of this enzyme requires higher concentrations of LH and human ChG [32]. The treatment of cells with the compound Org 43553 at concentrations of 10^{-6} and 10^{-5} M resulted in an increase of PLC activity to 33–37%, which is less than 5% of the corresponding effect of LH. These data indicate that the binding of LH/ChG-R with Org 43553 has a little effect on ability of this receptor to interact with G_q proteins and activate phosphoinositide turnover [31].

The Org 43553 was active in the in vivo conditions, including the oral administration [33, 34]. A single oral administration of Org 43553 at a dose of 50 mg/kg induced the ovulation in immature mice and adult rats. Ovulated oocytes were characterized by high fertility, and their implantation resulted in the formation of normal embryos. Oral administration of Org 43553 at the same dose to adult male rats increased the plasma level of testosterone. The study of pharmacokinetics of Org 43553 showed that, in comparison with gonadotropins, this compound degrades more quickly [33]. A decrease in the half-life has a great practical importance, since it allows reducing the risk of ovarian hyperstimulation syndrome, one of the most severe complications of gonadotropin-stimulated ovulation. A single oral administration of
Org 43553 to female rats had no effect on the size of ovaries and on the permeability of ovarian blood vessels, and even multiple treatments of animals with Org 43553 did not result in the development of ovarian hyperstimulation syndrome [34]. The cause of this is that the compound Org 43553 reduces the level of vascular endothelial growth factor, a crucial inducer of vascular permeability, while gonadotropins increase the concentrations of this factor. At a dose of 300 mg, Org 43553 induced ovulation in 83% of women of reproductive age. In the case of experimental animals, the signs of ovarian hyperstimulation syndrome were not observed, and this fact indicates good prospects for the use of Org 43553 in the induction of ovulation in clinics [35].

We studied new analogs of Org 43553, the compounds TP01 and TP02, which were synthesized by acylation of thienopyrimidine precursor at the 5-amino group using isonicotinoyl and thiophene-3-carbonyl chlorides, respectively. Both compounds were active in the in vitro conditions, stimulating ACSS in the reproductive tissues [36, 37], and in the in vivo, increasing testosterone level in the case of different mode of drug administration to male rats [38]. The TP01 and TP02 stimulated the basal AC activity in plasma membranes isolated from the testes and ovaries of rats, and the EC50 values for their effects on AC activity amounted to 1.05–1.12 mM and 280–365 nM, respectively. Along with this, in testicular membranes, the TP01 and TP02 increased GTP-binding capacity of Gs proteins [36]. The AC stimulating effect of human ChG in the presence of thienopyrimidine derivatives was retained, and at low, nonsaturating concentrations, the additive effect of human ChG and TP01/TP02 was observed, indicating different localization of gonadotropin- and thienopyrimidine-binding sites in LH/ChG-R [37].

In the in vivo experiments on rats, the compound TP01 administered intraperitoneally at a dose 15 mg/kg after 3 and 5 h increased the plasma testosterone level by 83 and 339% and at a dose 27 mg/kg—by 134 and 325%, respectively. The increase of testosterone level 3 h after TP01 treatment at the doses 15 and 27 mg/kg was 13 and 21% of that induced by human ChG (i.p., 250 IU/rat). Meanwhile, the TP01 action was more prolonged, and 5 h after injection, the TP01-induced increase of testosterone level was 44–46% of that of human ChG [38]. It is important that TP01 was active when given orally, and at a dose of 50 mg/kg, it increased testosterone level by 230 (3 h) and 417% (5 h). The TP02 was less active when administered orally, which is probably due to reduced ability to achieve the Leydig cells as a result of rapid degradation of TP02 or its impaired absorbability in the gastrointestinal tract.

Recently, we synthesized another thienopyrimidine derivative, 5-amino-N-tert-butyl-2-(methylsulfanyl)-4-(3-(nicotinamide)phenyl)thieno[2,3-d]pyrimidine-6-carboxamide (TP03) and showed that TP03 at a concentration of 10−4 M stimulated AC activity by 213% in rat testicular membranes, and the EC50 value for its effect was 390 nM [39]. The TP03 when administered intraperitoneally (25 mg/kg) and orally (50 mg/kg) significantly increased the plasma testosterone level in male rats, and in this respect, it was more effective than TP01. These data indicate that TP03 can be used to develop the drugs regulating the steroidogenesis in Leydig cells.

In the case of TSHR, the most important task is the development of LMW compounds with activity of inverse agonists and neutral antagonists that inhibit the basal and hormone/antibody-stimulated activity of TSHR, respectively [40]. It was found that in Graves’ disease the TSHR-stimulating antibody causes hyperstimulation of the thyroid gland, which leads to...
dysfunction of hypothalamic-pituitary-thyroid axis [41]. Nowadays, the effective methods for treating hyperthyroidism were not developed. The approaches that are commonly used are invasive and effective only in treating the complications of hyperthyroidism, without affecting the causes of thyroid pathology.

In 2008, Susanne Neumann and colleagues developed highly selective TSHR neutral antagonist NIDDK/CEB-5 that is structurally close to Org 43553 [42]. The treatment of TSHR-expressing HEK-EM 293 cells with 30 μM of the compound NIDDK/CEB-52 significantly decreased effects of TSH and TSHR-stimulating antibody on AC activity, and the IC\textsubscript{50} value for inhibitory effect of this compound was 4.2 μM. The 24-h incubation of thyrocytes with TSH and NIDDK/CEB-52 (10 μM) led to a threefold decrease in TSH-induced expression of the gene encoding thyroid peroxidase that is required for iodination of tyrosine residues on thyroglobulin and, thereby, controls the production of thyroid hormones. In 2011, a potent TSHR inverse agonist NCGC00229600, 2-(3-(2,6-dimethylphenoxy)methyl)-4-metoxyphenyl)-3-(pyridin-3-ylmethyl)-2,3-dihydroquinazolin-4(1H)-one, was developed, and it, unlike NIDDK/CEB-52, suppressed both basal and stimulated TSHR activity [43]. The NCGC00229600 decreased effect of TSHR-stimulating antibody on AC activity by 30–75% in human thyrocytes and in the primary culture of fibroblasts obtained from the retro-orbital space of patients with Graves’ disease [43, 44]. The ability of NCGC00229600 to suppress TSHR activity in retro-orbital fibroblasts is very important because AC overstimulation in these cells induced by TSHR-stimulating antibody is one of the main causes of ophthalmopathy, severe symptom of Graves’ disease.

Newly developed neutral furan-containing antagonist NCGC00242364 selectively inhibited TSH-induced AC activity in TSHR-expressing cells and suppressed the effects of TSH and TSHR-stimulating antibody on thyroxine production and expression of the genes encoding Na\textsuperscript{+}-I\textsuperscript{-} cotransporter and thyroid peroxidase in female mice BALB/c [40]. These data demonstrate high efficiency of the NCGC00242364 and its analogs in the treatment of Graves’ disease. It is very important that these compounds had no effect on the basal TSHR activity, which allows avoiding the development of hypothyroid states typical for TSHR inverse agonists [40]. The inverse agonists are more suitable to treat nonautoimmune hyperthyroidism caused by activating mutations in TSHR.

The development of LMW ligands of LH/ChG-R and TSHR allows creating a wide range of drugs for selective regulation of hypothalamic-pituitary-gonad and hypothalamic-pituitary-thyroid axes. The LMW agonists of LH/ChG-R can be used to induce ovulation and fertilization in females and to enhance the production of steroid hormones in males with androgen deficiency and hypogonadotropic states. The inverse agonists and neutral antagonists are useful for contraception and treatment of hormone-dependent tumors of the reproductive system. The LMW antagonists and inverse agonists are the promising drugs for prevention of hyperactivation of the thyroid gland by TSH and TSHR-stimulating antibody, while the full TSHR agonists can be used in the diagnostics of thyroid cancer and in radioiodine therapy. It should be noted that LMW regulators are active when administered orally because they do not degrade in the gastrointestinal tract and are easily absorbed into blood. They are effective even when they act on mutant forms of GPCRs that are not capable to processing and translocation into the plasma membrane. The LMW agonists act as highly selective pharmacological chaperones for the cognate receptors. The agonists penetrate into the cells, bind to mutant
GPCRs located in the cytoplasm or endoplasmic reticulum, stabilize their active conformation, preventing receptor degradation in proteasomes and facilitating GPCR translocation into the plasma membrane [45, 46].

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References

[1] Luttrell LM. Transmembrane signaling by G protein-coupled receptors. Methods in Molecular Biology. 2006;332:3-49. DOI: 10.1385/1-59745-048-0:1


[17] Shpakov AO, Shpakova EA, Tarasenko II, Derkach KV. Receptor and tissue specificity of the effect of peptides corresponding to intracellular regions of the serpentine type

[18] Forsman H, Bylund J, Oprea TI, Karlsson A, Boulay F, Rabiet MJ, Dahlgren C. The leukocyte chemotactic receptor FPR2, but not the closely related FPR1, is sensitive to cell-penetrating pepducins with amino acid sequences descending from the third intracellular receptor loop. Biochimica et Biophysica Acta. 2013;1833:1914-1923. DOI: 10.1016/j.bbamcr.2013.03.026


[37] Shpakov AO, Derkach KV, Dar’in DV, Lobanov PS. Activation of adenylyl cyclase by thienopyrimidine derivatives in rat testes and ovaries. Cell and Tissue Biology. 2014;8:400-406. DOI: 10.1134/S1990519X14050071

[38] Derkach KV, Dar’in DV, Lobanov PS, Shpakov AO. Intratesticular, intraperitoneal, and oral administration of thienopyrimidine derivatives increases the testosterone level in male rats. Doklady Biological Sciences. 2014;459:326-329. DOI: 10.1134/S0012496614060040


[41] Rapoport B, McLachlan SM. The thyrotropin receptor in graves’ disease. Thyroid. 2007; 17:911-922. DOI: 10.1089/thy.2007.0170


