Allosteric Modulation of 'Reproductive' GPCRs : a case for the GnRH and LH receptors

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CHAPTER 1

GENERAL INTRODUCTION

This chapter introduces G protein-coupled receptors (GPCRs) and the recent developments in drug research involving these proteins. Allosteric modulation will be discussed in more detail and especially its therapeutic potential for the 'reproductive' GPCRs, the GnRH and LH receptor. Finally, the scope and content of this thesis will be introduced.

1.1 G PROTEIN-COUPLED RECEPTORS

1.1.1 Introduction

The first primary structure (i.e. the protein sequence) of a G protein-coupled receptor (GPCR) was described twenty-five years ago, for bovine rhodopsin.[1] It was shown that activation of this membrane protein by light, an extracellular signal, resulted in an intracellular response. Since that time the structural elucidation of the GPCR receptor family further expanded with the aid of different molecular biological techniques. Completion of the human genome project provided a list of possible GPCR family members.[2] In 2007, it was reported that the human GPCR family consisted of 799 unique full-length members.[3]

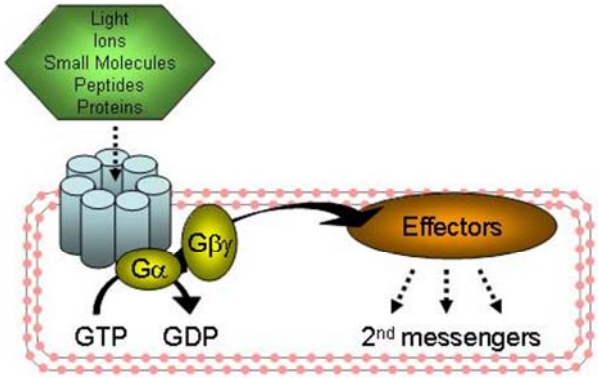

Figure 1.1 A simplified model of ligand binding to and activation of a GPCR.

The endogenous ligands for these receptors are very divergent, consisting of light (rhodopsin), cations (e.g. calcium-sensing receptor), small organic compounds (e.g. adenosine receptor), peptides [e.g. gonadotropin-releasing hormone (GnRH) receptor] or proteins [e.g. luteinizing hormone (LH) receptor] as shown in Figure 1.1.[4] Activation of the receptor by an extracellular ligand induces a conformational change that is followed by G protein binding to the intracellular loops and C-terminus of the receptor (Figure 1.1).[5] GTP replaces GDP on the Gα subunit, the G protein subunits (α and βγ) dissociate from the receptor and bind to their downstream effector proteins, e.g. adenylate cyclase (via $G\alpha_s$ or $G\alpha_i$) or phospholipase C (via $G\alpha_q$). In this way different cellular functions are controlled, such as growth, movement and gene expression. However, more and more evidence is accumulating that GPCRs can also signal independently from the G protein, which will be discussed in Chapter 1.1.3.[6]

1.1.2 GPCR Structure and Classification

All GPCRs contain seven transmembrane (7-TM) helices connected by three extracellular and three intracellular loops with an extracellular N-terminus and an intracellular C-terminus.

GPCRs can be divided in five groups according to a phylogenetic classification; Rhodopsin-like (class A), Secretin receptor-like (class B), Glutamate receptor-like (class C), Frizzled receptors and Adhesion-like receptors.[7] However, usually a division into four groups is made; class A, B, C (Figure 1.2), where Adhesion-like receptors are part of class B, and a fourth Frizzled receptor class.

The class A family of GPCRs is the largest and consists of approximately 670 members.[3] More than half of class A GPCRs are olfactory receptors, which are activated by a broad range of odorants.[8] Although, they are interesting targets for the fragrance industry, these receptors are usually not considered as drug targets. In general, the N-terminus of class A GPCRs is relatively short and an additional eighth helix is present at the C-terminus of the receptor (Figure 1.2). The binding site of the endogenous ligand, termed the orthosteric binding site, is often located within the 7-TM domain of the receptor. Ligands for these receptors are widely varied, consisting of amines, peptides and lipids. The secretin-like GPCR family (class B) is much smaller than class A consisting of nearly 50 members.[4] These receptors have a large extracellular N-terminus containing several conserved cysteine bridges, which results in a rigid structure (Figure 1.2). The endogenous ligands are peptide hormones, such as calcitonin, glucagon and parathyroid hormone that bind to the extracellular domain of the receptor. Class C or glutamate-family of GPCRs contains a large N-terminus, similar to class B receptors (Figure 1.2). However, an additional unique motif is present, named the Venus flytrap, consisting of two domains that form a cavity where the endogenous ligand binds.[9]

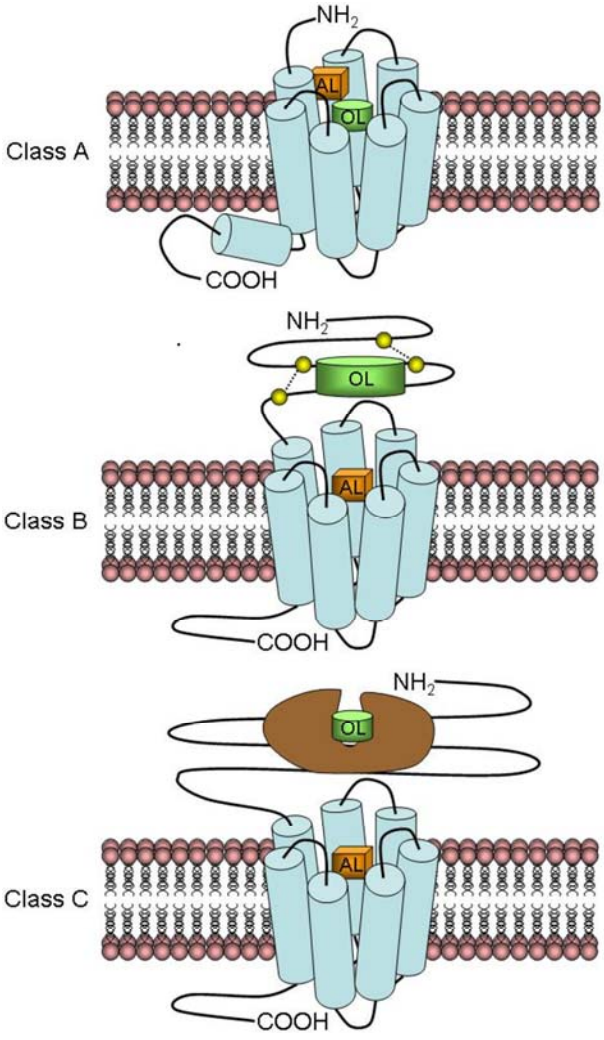

Figure 1.2 Schematic representations of the structure and binding pocket of orthosteric (OL) and allosteric ligands (AL) for class A, B and C GPCRs.

1.1.3 'Reproductive' GPCRs

GnRH Receptor. The GnRH receptor is classified as a class A receptor based on its sequence homology of the 7-TM domain.[4] It contains the typical short N-terminus followed by seven α-helical bundles. However, a unique feature of the human GnRH receptor is that it lacks the C-terminus.[10] The GnRH receptor is predominantly coupled to $G\alpha_q$, however, interactions with other G proteins have been reported.[11] As the human GnRH receptor does not have an intracellular C-terminus, interactions with multiple G proteins should occur via the intracellular loops.[10] Class A GPCRs are further divided into four groups, α, β, γ and δ, in which the GnRH receptor belongs to the β-group.[12] All known ligands for this group of receptors are peptides. In this case the endogenous ligand is GnRH, a decapeptide that is produced in the hypothalamus.[10] As shown in Figure 1.2 for class A GPCRs, the binding pocket of the orthosteric ligand, GnRH, is located within the 7-TM domain. Most peptidic ligands for GPCRs also interact with amino acids in the extracellular loops and exofacial parts of the 7-TM domain.[13] In the past decade, several non-peptidic and low molecular weight (LMW) antagonists for the human GnRH receptor have been reported.[14] These (and other) compounds and their putative binding pocket will be discussed in *Chapter 2*.

LH Receptor. Based on the GPCR classification described in Chapter 1.1.2 and as shown in Figure 1.2 the human LH receptor could easily be classified as a class B GPCR. Like class B GPCRs, the LH receptor contains a large N-terminus which binds the endogenous ligand, the glycoprotein hormone LH. This receptor, however, has been classified as a class A GPCR based on conserved amino acid motifs in its 7-TM domain.[4] Most rhodopsin-like GPCRs have a short N-terminus without any conserved domains. However, there are some exceptions within class A, namely the leucine-rich-repeat-containing GPCRs (LGRs)[15-17] and the glycoprotein hormone receptors.[18] These eight receptors, LGR4-8, follicle-stimulating hormone (FSH) receptor, luteinizing hormone (LH) receptor and thyroid-stimulating hormone (TSH) receptor, have in common that they contain several leucine-rich domains

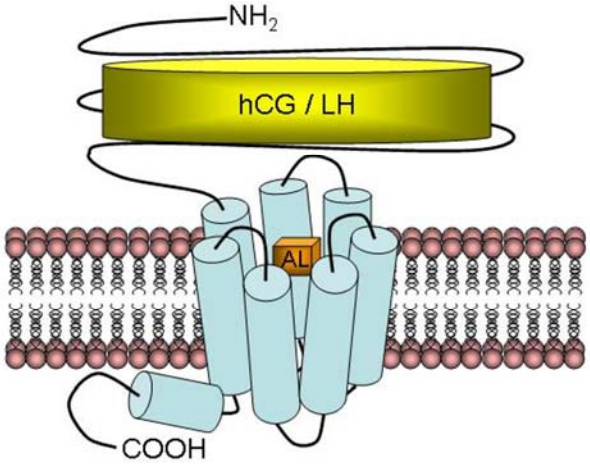

Figure 1.3 Schematic representations of the structure and binding pockets of orthosteric (hCG and LH) and allosteric ligands (AL) of the LH receptor.

in their large N-terminus. These receptors belong to the δ-group of class A GPCRs and their endogenous ligands are peptide hormones, relaxin (LGR7 and 8), or glycoprotein hormones (FSH, LH and TSH receptor) that bind to this N-terminal domain (Figure 1.3). Notably, LGR4, 5 and 6 are still orphan receptors according to the IUPHAR database, i.e. the endogenous ligand is not known.[4,19] Activation of the LH receptor predominantly results in cAMP production via $G\alpha_s$. Activation of the LH receptor is thought to occur when hCG or LH bind to the N-terminus, which causes the 'hinge-region' (i.e. amino-acids connecting 7-TM to N-terminal domain) to interact with the 7-TM domain, leading to receptor activation.[20] Recently, it was shown that not only the high molecular weight (HMW) endogenous ligands are able to do that, but also LMW ligands.[21] These LMW ligands bind to the 7-TM domain (the allosteric site) of the receptor similar to where most other ligands of class A GPCRs bind (Figure 1.3). These (and other) LMW ligands will be discussed in *Chapter 2*.

1.1.4 Current Developments in Drug Discovery for GPCRs

Currently more than 30% of the marketed drugs target GPCRs.[22] However, the general trend in drug development has been towards increasing research and development (R&D) costs and decreasing output; the number of novel drugs approved by the FDA is depressingly low, especially from 1996 onward (Figure 1.4).[23,24] Therefore, it is necessary that the GPCR research field develops novel approaches for drug discovery. One of these so-called 'hot topics' is *allosteric modulation*, which will be discussed in more detail in Chapter 1.2. First, other important developments in the field dealing with novel concepts, novel targets and a basic understanding of receptor structure will be briefly discussed.

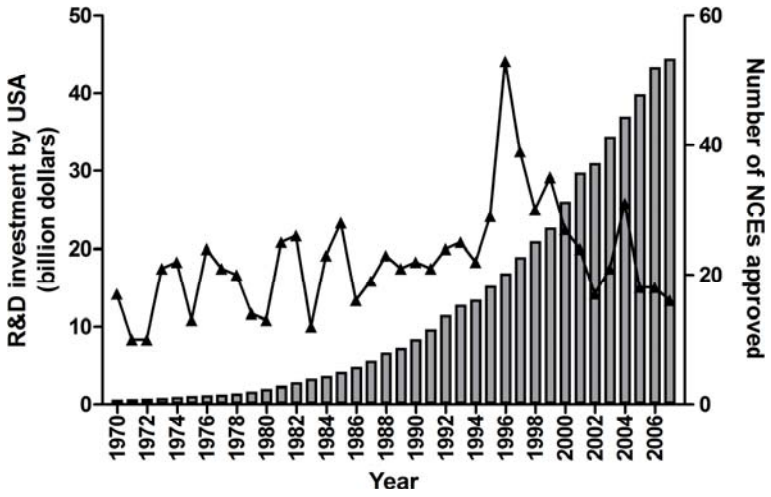

Figure 1.4 The bargraph and line show the R&D investments and number of NCEs approved by the FDA from 1970 to 2007, respectively.

Constitutive Activity. The first report of a constitutively active receptor, i.e. a receptor that is active in the absence of an agonist, already dates back to the late eighties.[25] Somewhat later the group of Lefkowitz showed that a single mutation in the α_{1b}-adrenergic receptor resulted in a constitutively active receptor.[26] Since then many mutation-induced constitutively active receptors have been reported and the therapeutic potential of inverse agonists, i.e. ligands with negative intrinsic efficacy, has been reviewed.[27,28] However, the importance of constitutive activity *in vivo* is still poorly understood, as the presence of an endogenous (orthosteric or allosteric) ligand can often not be ruled out.[29]

Receptor Dimerization. Rhodopsin is arranged as oligomers in native disc membranes.[30] This has been taken as evidence that class A GPCRs can occur in dimers or oligomers of higher order. However, the expression levels of rhodopsin are this high that oligomerization might be inevitable. There is conclusive evidence for other class A GPCRs, such as the $GABA_B$ receptor,[31] and mu and delta opioid receptors,[32] where the formation of heterodimers can result in distinct pharmacology. Most reports on homo- or heterodimerization of GPCRs result from co-expression of receptors in heterologous cell systems, and can thus not be taken as proof for the physiological occurrence of receptor dimers. The field of receptor dimerization could have great potential in drug discovery, especially if ligands could be developed that are dimer-selective.[33]

Ligand-Directed Signaling. Recently, it was shown that GPCRs can function G protein-independently. For example, β-arrestin, which was already known for its role in agonist-induced receptor internalization, or tyrosine kinase Src can have important direct signaling functions.[34,35] GPCRs are, therefore, more often referred to as 7TM or serpentine receptors. Another development is the possibility of functional selectivity or biased-agonism. In this case, the activation of a certain signaling pathway is directed by the ligand that activates the receptor. For example, binding of the endogenous ligand, LH, to its receptor activates both the cAMP pathway and the PLC pathway. However, the LMW agonist, Org 43553, only activates the cAMP pathway.[36] In addition, it was shown for the β_2-adrenoceptor different stereo-isomers of a ligand can activate a different signaling pathway.[37] This shows the need for analyses of multiple pathways in order to find new ligands or to understand the effects of a certain ligand.

'Deorphanization'. In class A-C of GPCRs there are receptors that have unknown endogenous ligands (orphan receptors). According to the IUPHAR database, approximately 120 orphan receptors still need to be linked to a ligand.[19] It is thought that some of these

receptors might not even have an endogenous ligand, but modulate the functions of other proteins by dimerization, constitutive activity or other mechanisms.[38] For example, GPR50 was shown to form a heterodimer with the melatonin MT1-receptor, resulting in strongly decreased melatonin binding.[39] 'Deorphanization' or elucidation of ligand-independent functions of these receptors could possibly yield new drug targets.

Receptor Crystallization. The first GPCR crystal structure, rhodopsin, was obtained in 2000.[40] It proved to be much more difficult to crystallize other GPCRs and it took seven years before another crystal structure, the human β_2-adrenergic receptor, was published.[41,42] Two different methods were used to obtain conformational stability next to the presence of the partial inverse agonist carazolol with high affinity. Firstly, a monoclonal antibody (Mab5)[42] was generated against the third intracellular loop or secondly, T4 lysozyme (T4L)[41] was inserted in the third intracellular loop. The presence of the inverse agonist in combination with either Mab5 or T4L resulted in a less flexible receptor, thereby facilitating crystallization. Another method of crystallization, i.e. constraining the receptor, was reported a year later for the (turkey) β_1-adrenergic receptor.[43] Random mutagenesis was performed on the receptor to increase the thermostability of the receptor. In the presence of an antagonist, cyanopindalol, the engineered thermostable β_1-adrenergic receptor was stabilized to a single conformation, a prerequisite for crystallization. Similarly, two classes of engineered thermostable adenosine A_{2A} receptors were reported for either agonist or antagonist occupancy.[44] Comparison of the applied mutations does not point to a general amino acid pattern to increase thermostability. More recently, the human adenosine A_{2A} receptor crystal structure was obtained in combination with a high affinity antagonist, ZM241385, and the T4L method.[45] Surprisingly, the binding pocket of this antagonist differed greatly from carazolol in the β_2-adrenergic receptor. This indicates that one should be cautious in interpreting results from molecular modeling and ligand docking studies based upon existing (inactive) crystal structures. Notably, a welcome addition to these 'inactive' crystal structures would be the crystal structure of an active conformation of a GPCR (i.e. a receptor with an agonist). Until then several questions remain, such as what the active state of the receptor looks like and if this state is the same for each type of ligand (e.g. protein or LMW).

1.2 ALLOSTERIC MODULATION

1.2.1 Allosteric Modulation of GPCRs

Allosteric modulation was first reported for enzymes. In the field of enzymology it was noted that the chemical structure of inhibitors was often very different from the substrate of the enzyme. Therefore, it was suggested that another binding site accommodated these inhibitors through which they transmitted their effect to the substrate site.[46] GPCRs, however, are naturally modulated by the presence of the (allosteric) G protein.[47] GPCRs can interact with a variety of other cellular proteins that, for example, influence receptor activation.[48] Moreover, much smaller molecular entities have been reported as endogenous allosteric modulators for GPCRs, such as (cat)ions, peptides, and lipids.[49] For example, Zn^{2+} and anandamide have been shown to allosterically inhibit dopamine D_2 receptors[50] and M_1 muscarinic acetylcholine receptors,[51] respectively. Interestingly, endogenous allosteric modulators also play a role in some autoimmune diseases. For example in Sjögren's syndrome in which (allosteric) autoantibodies are raised against M_3 muscarinic acetylcholine receptors, thereby enhancing their activity.[52]

For class A GPCRs the classical orthosteric site is accommodated by helices III, V and VI, and to some extent helix VII. This pocket can be accessed by low molecular weight ligands, which is supported by the recent crystal structures of the β-adrenergic receptors[41,43] and adenosine A_{2a} receptor[45] with ligand bound. As schematically represented in Figure 1.2, the allosteric binding site for class A GPCRs is most likely located in the 7-TM domain as well. For the GnRH receptor, it was shown recently that the allosteric site partially overlaps with the orthosteric site.[53] The orthosteric site of the glycoprotein hormone receptors is located at the large N-terminus, which results in an essentially unoccupied 7-TM domain (Figure 1.3). Experiments with chimeric receptor constructs for the LH[36] and FSH[54] receptor have indeed shown that allosteric ligands bind in that domain. However, it seems that two different sites are occupied here, i.e. the classical class A orthosteric site and a second smaller pocket that is formed by helices I, II, III and IV.[55]

1.2.2 Detecting and Describing Allosteric Modulation

In the last two decades, methods for the identification of new ligands were based on equilibrium displacement assays using a radiolabeled or otherwise tagged (orthosteric)

ligand. As a consequence, new ligands were often orthosteric of nature as (true) allosteric ligands do not compete with the radioligand. For clarity, the orthosteric binding site is referred to as the site which binds the endogenous ligand, while the allosteric site is a topographically distinct binding site (Figure 1.2).[56] Nowadays, functional assays are used in high-throughput screens (HTS) to find new allosteric ligands for certain drug targets, e.g. GPCRs.[57] In addition, kinetic association and dissociation assays of the (radio)ligand-receptor interaction are often used. The binding of an allosteric ligand induces a conformational change in the receptor, thereby altering the rates at which the orthosteric ligand associates or dissociates from its binding site.[58] With the aid of these different screening methods, allosteric modulators have been reported for all classes (A-C) of GPCRs.[49] Therefore, allosteric modulation of GPCRs seems to be a rule rather than an exception.

Several mathematical models have been developed that describe different ligand-receptor interactions.[58] One of the first and most simple models is the linear two-state model (Figure 1.5a).[59] This model uses an equilibrium dissociation constant (K_A) to describe the interaction between a ligand (A) and a receptor (R). It proposes that ligand binding results in a conformational change of the receptor from an inactive to an active state. The active receptor conformation will ultimately elicit a biological response. This model was not sufficient to explain experimentally obtained data on allosteric modulation. Therefore, the allosteric ternary complex model was developed (Figure 1.5b).[60] In this model the effect of the binding of an allosteric ligand (B) on the affinity (α) and efficacy (β) of an orthosteric ligand (A) is described. However, next to 'true' allosteric modulators, i.e. compounds that do not have an intrinsic activity on their own, allosteric agonists have been identified. These compounds are able to activate the receptor by binding at an allosteric site. Addition of this possibility and constitutive receptor activity into a new model resulted in the allosteric two state

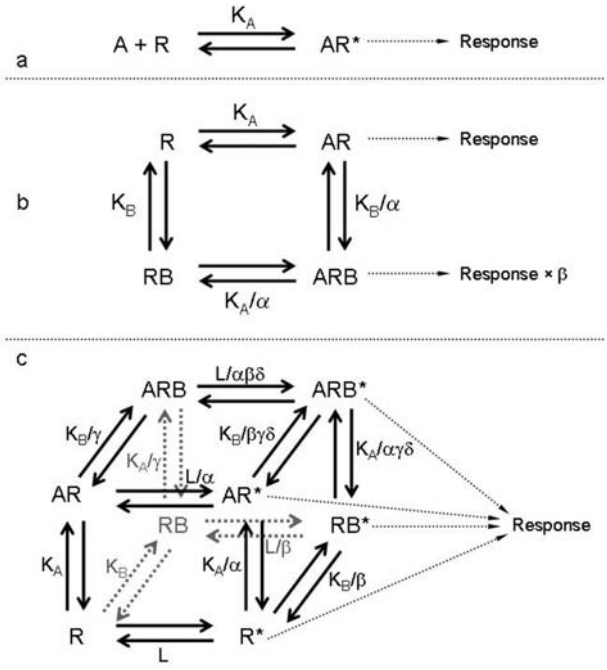

Figure 1.5 Mathematical models that describe ligand-receptor interactions. a) The linear two-state model. b) The allosteric ternary complex model. c) The allosteric two-state model.

model (Figure 1.5c).[61] In this model orthosteric and allosteric ligands can bind and activate the receptor, the extent of which is described by the cooperativity factors α and β, respectively. The ability of the ligands to modulate the binding of and activation by each other is described by cooperativity factors γ and δ, respectively. Moreover, constitiutive activity of the receptor is taken into account (L). Extension of these models is possible for accommodation of multiple allosteric sites,[62] G protein-coupling [58] or allosteric modulation across dimeric receptors.[63] Notably, the cooperativity factors shown in Figure 1.5 can be different for each orthosteric-allosteric ligand pair at a given receptor, also referred to as probe dependence.[49] Hence, the physiological relevance of a certain allosteric effect should always, when possible, be examined with a physiologically relevant probe, the endogenous ligand.

1.2.3 Therapeutic Potential – Allosteric Modulation

Most drugs targeting GPCRs that are currently on the market are orthosteric in nature.[64] For a therapeutic effect these compounds must have a high affinity for the orthosteric site and a high local concentration should be maintained. The resulting disadvantage of synthetic orthosteric ligands are therefore effects such as, toxicity, desensitization and long-term changes in receptor up/down regulation.[49] Allosteric modulators have the potential to overcome these negative effects. Moreover, these compounds have other advantages that orthosteric ligands do not possess, which will be described in more detail below. Therefore, allosteric modulation of GPCRs has fuelled further interest by scientists from academia and industry.

Only two allosteric modulators of GPCRs are currently on the market, Cinacalcet and Maraviroc, which are an allosteric enhancer of the calcium-sensing receptor[65] and an allosteric inhibitor of the CCR5 receptor,[66] respectively. LMW allosteric ligands potentially have several advantages over orthosteric ligands.[67] The main advantage results from the fact that allosteric ligands target a different binding site than the endogenous (orthosteric) site. This site has not been conserved through evolution, which results in increased receptor subtype selectivity. For example, the adenosine receptors consist of four subtypes (A_1, A_{2a} A_{2b}, A_3) that all bind the endogenous ligand adenosine due to a conserved (orthosteric) binding pocket. For these (and other) receptors it was shown that allosteric modulators can be more selective than synthetic orthosteric ligands.[68] Allosteric modulators are characterized as

compounds that only have an effect in the presence of the endogenous ligand. The latter yields two additional advantages, saturability of the effect and preservation of physiological patterns. Firstly, increasing the dosage of an allosteric modulator per se will not produce an increased effect, also known as the 'ceiling-effect'. The cooperativity factor of the allosteric modulator and the orthosteric ligand determines the effect. In addition, allosteric modulators with a lower affinity can be administered at a higher dose with less safety or toxicity problems. Secondly, the allosteric modulator will only exert an effect where and when the endogenous ligand is produced. This results in tissue selectivity and the duration of an effect remains physiologically relevant.

1.2.4 Therapeutic Potential – GnRH and LH Receptor

An additional advantage of allosteric modulation is worth mentioning for GPCRs (e.g. GnRH and LH receptor) that have peptide or protein hormones as endogenous ligands, which lack oral bioavailability. Synthetic ligands for these receptors (both orthosteric and allosteric) can be made drug-like, i.e. LMW, orally bioavailable, metabolically stable and with an acceptable safety profile. In addition, pure synthetic ligands lack batch variability and contamination with other proteins, when compared to proteins obtained from urine or recombinant production.[69] Examples of drug-like ligands for the GnRH and LH receptor are shown in Figure 1.6. The chemical structures and size of these LMW ligands are compared to the crystal structures of GnRH (PDB entry: 1yy1) and hCG (PDB entry: 1hrp). NBI-42902 is an (orthosteric) antagonist for the GnRH receptor (Figure 1.6a), which has been shown to suppress plasma LH levels after oral administration in post-menopausal women.[70] For the LH receptor, Org 43553 was introduced as the first potent and orally active allosteric agonist that induced ovulation in mice and rats.[69]

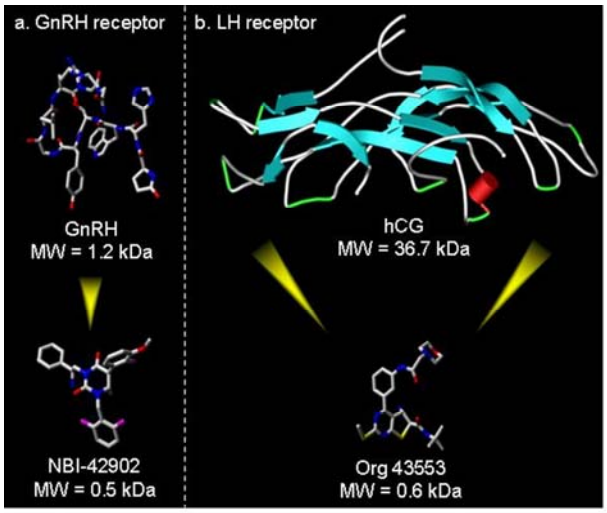

Figure 1.6 Structural comparisons of the endogenous HMW ligand and a LMW ligand for the (a) GnRH and (b) LH receptor, respectively.

The GnRH receptor has been described as a potential target for different diseases. So far, orthosteric (peptide) agonists and antagonists are well-characterized. Allosteric enhancers and inhibitors could be beneficial in similar treatments. GnRH receptor agonists and antagonists have been shown to be efficacious in IVF procedures. It should be noted that (peptide) agonists are used to desensitize the receptor, which in turn also results in a decreased gonadotrope function.[71] In addition, GnRH receptor ligands may also be applied in a number of sex hormone-dependent conditions.[72,73] Notably, various peptide GnRH receptor agonists and antagonists are marketed for the treatment of prostate, breast, uterine and ovarian cancer, leiomyomas, infertility, benign prostatic hyperplasia (BPH), IVF, premenstrual syndrome and endometriosis.[74,75]

The LH receptor is an important regulator of reproductive functions in humans. Currently, recombinant LH (recLH) is used for the treatment of female hypogonadism.[71] In addition, recLH was approved for the use in the late follicular phase of IVF treatment to enhance oocyte maturation and pregnancy outcome.[76] Similarly, recombinant hCG and urinary hCG are used for ovulation induction and oocyte maturation. However, these hormone preparations need to be administered daily by subcutaneous injection. Therefore, efforts are made to develop more patient friendly formulations, such as gonadotropins with longer half-lives[77,78] and orally bioavailable drugs.[55] Another important goal is to eliminate ovarian hyperstimulation syndrome (OHSS), a side effect resulting from the hormonal treatments.[79] GnRH receptor agonists already show improvement, however, they are peptidic in nature. Therefore, LMW LH receptor agonists are of interest here. Recently, it was shown that such a compound, the allosteric agonist Org 43553 can possibly be used for ovulation induction or final oocyte maturation in IVF therapy with reduced side effects (e.g. OHSS).[69] Gonadotropins (hCG) have also been shown to promote ovarian tumor cell growth[80] and inhibit primary breast tumor growth.[81] Thus, negative and positive allosteric modulators could be beneficial in these cases, respectively. Notably, several naturally occurring mutations have been described for the LH receptors that are involved in male and female fertility.[82] These data indicate that LH receptor antagonists or allosteric inhibitors could be useful as contraceptives.

1.3 THIS THESIS

In the previous paragraphs GPCRs, the concept of allosteric modulation and the therapeutic potential thereof for the human GnRH and LH receptor were presented. In earlier work we have demonstrated that other class A GPCRs (adenosine A_1, A_{2A} and A_3 receptors) can be allosterically modulated.[68] In this thesis we have expanded our knowledge on allosteric modulation of class A GPCRs to two of the 'reproductive' family members, the GnRH and LH receptor. As both these receptors have protein hormones as endogenous ligands, new LMW ligands (either allosteric or orthosteric) are of general interest. Therefore, a review of the current literature on LMW ligands for GPCRs of the hypothalamic-pituitary-gonadal axis (HPG) (e.g. GnRH and LH receptor) is presented in *Chapter 2*.

At the start of this project, no allosteric modulators of GnRH and LH receptors were known. Therefore, the effect of general allosteric modulators (e.g. GTP, sodium ions and amiloride derivatives) on dissociation kinetics of either GnRH or LH receptor ligands was examined. For the GnRH receptor we found that an amiloride analog (HMA) was a potent allosteric inhibitor. Meanwhile, Sullivan and coworkers reported that a non-peptidic antagonist for the human GnRH receptor also displayed allosteric effects.[53] Intrigued by the availability of two allosteric ligands [HMA and FD-1 (an analog of Sullivan's compound)], the studies described in *Chapter 3* were performed. Figure 1.7 schematically represents the question that needs to be answered when three structurally different ligands are available for one receptor. In this case the green, red and cyan ligands represent GnRH, HMA and FD-1 binding at the human GnRH receptor (blue), respectively.

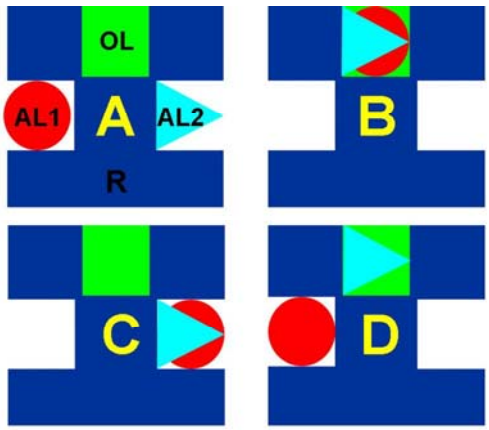

Figure 1.7 Four different possibilities of ligand distribution (A-D) in the receptor (R), when the orthosteric ligand (OL; square) and two other (allosteric) ligands (AL1; circle and AL2; triangle) are present.

In 2002, the first orally active LMW agonist for the LH receptor was reported.[21] A more potent analog was labeled with tritium and *Chapter 4* introduces this first LMW radiolabeled agonist for the human LH receptor, [^{3}H]Org 43553. We hypothesized that similar to other class A GPCRs, the LH receptor could possibly also contain two binding sites in the 7-TM domain. Therefore, a screen was performed in which allosteric modulation of [^{3}H]Org 43553 was examined. This resulted in the first class of allosteric inhibitors (e.g. LUF5771; *Chapter*

5) and allosteric enhancers (e.g. LUF5419; *Chapter 6*). When Figure 1.7 is used to describe the LH receptor (blue), the green, red and cyan ligands represent LH (or hCG), Org 43553 and a LUF compound (e.g. LUF5419 or LUF5771), respectively. Moreover, the same library of compounds was screened for an inhibitory effect in a reporter gene assay, more specifically a luciferase assay (*Chapter 7*). This resulted in a surprising amount of apparent LH receptor antagonists. We felt that this deserved some more attention and it appeared that some of these compounds were competitive inhibitors of the enzyme rather than of the receptor.

In conclusion, in this thesis I present the evidence that 'reproductive' GPCRs, like most other class A GPCRs, can be allosterically modulated by LMW ligands. *Chapter 8* will conclude this work, describing the general conclusions and future perspectives for this field of research.