CHAPTER 5

MATHEMATICAL MODELLING OF NONPEPTIDE ANTAGONIST TO THE HUMAN GONADOTROPIN RELEASING HORMONE RECEPTOR

5.1 Introduction

A Mathematical model is developed to describe the hormonal interaction of the human gonadotropin-releasing hormone (GnRH). These findings are of importance for the future definition of nonpeptide ligand structure–activity relationships and for the identification of potentially useful slowly dissociating antagonists for the GnRH receptor. Numerical solution of the models yield results that periodic and in good qualitative agreement with physiological observations **[9].**

The chief aim of this study is to construct a mathematical model which is notably simpler than existing models, yet is able to describe the interaction of the key components and reproduces many of observed and the kinetic of uracil-series nonpeptide antagonist interaction with the GnRH receptor, utilizing a novel nonpeptide ligand. The principle findings are:

(a) Uracil-2 and Uracil-3 dissociated very slowly from the receptor;

(b) Differing substitutions at the 2-position of the benzyl group (at position 1 of the uracil) markedly affected the dissociation rate constant of the ligands without appreciably affecting the ligand association rate constant;

(c) This kinetic SAR was poorly resolved by measuring the apparent affinity in standard competition binding experiments, an effect that was quantitatively consistent with a lack of receptor ligand equilibration;

(d) The differences in dissociation rate constant Gonadotropin-releasing hormone (GnRH) is a linear decapeptide secreted from the hypothalamus into the portal circulation. GnRH binds to the GnRH receptor on gonadotropin cells in the anterior pituitary **[2, 26, 37].**

GnRH receptor activation stimulates the synthesis and release of luteinizing hormone (LH) and follicle stimulating hormone (FSH), which bind to gonadal receptors, stimulating the synthesis of the sex steroid hormones estrogen and testosterone **[49]**. The GnRH receptor is a seven transmembrane domain G-protein coupled receptor (GPCR) belonging to the class A family of GPCRs. The GnRH receptor signals redominantly through the GTP binding proteins Ga_{10} and Ga_{11}, stimulating phosphatidyl nositol turnover by phospholipase C, mobilization of intracellular calcium, diacylglycerol formation, protein kinase C activation, and arachidonic acid release **[95]**. GnRH

peptide agonists have been used therapeutically modulate the reproductive endocrine axis in a variety of disorders including precocious puberty, endometriosis, prostate cancer, uterine fibroids, breast cancer, and fertility disorders **[49].**

Overall, gonadal suppression is observed with GnRH agonist peptide therapy, an effect that results from desensitization and down regulation of pituitary GnRH receptors. Although GnRH agonist peptides have proven to be clinically effective, there are several drawbacks to this approach, including initial stimulation of the pituitary and subsequent increases in LH, FSH, and sex steroids prior to the GnRH receptor down regulation.

The hormonal flare seen with GnRH agonist peptides can be avoided by the use of GnRH peptide antagonists, such as abarelix and cetrorelix, which bind pituitary GnRH receptors without stimulating FSH and LH release. However, treatment with agonist and antagonist peptides requires parenteral administration and typically involves depot formulation. Consequently, considerable (Fig-5.1) effort has been directed towards development of orally active non-peptide GnRH receptor antagonists. Many novel small molecules have been synthesized to understand the structure activity relationships of antagonist binding affinities for the GnRH receptor.

However, little is known about the binding kinetics of these ligands to the GnRH receptor. Evaluation of receptor binding kinetics can provide an additional dimension for the understanding of ligand pharmacology .For example, measuring receptor–ligand binding kinetics of slowly equilibrating ligands can reveal structure–activity relationships (SAR) that can be masked or distorted in measurements of apparent equilibrium binding affinity ,owing to lack of equilibration of ligand with receptor in competition assays Slow antagonist dissociation from receptors can affect the mode of functional antagonism.

In vitro; this effect can reduce the maximal response of an agonist in functional antagonism experiments **[37]**. In vivo, slow receptor antagonist dissociation has been proposed to prolong and enhance antagonist efficacy, for example candesartan blockade of the angiotensin type 1 (AT1) receptor in rats and humans **[97,141]**. Here, the kinetic structure activity relationships of a series of nonpeptide antagonists is evaluated for the GnRH receptor, employing a novel nonpeptide ligand. This structure activity relationships was then used to investigate the relationship between the kinetics of ligand binding and the mode of functional antagonism of GnRH-stimulated cellular signaling **[110,132,145].**

5.2 Parameter interpretation

$\alpha_f \rightarrow$ Ligand Association rate constant

$\beta_f \rightarrow$Ligand Dissociation rate constant

$\gamma_f \rightarrow$ Unlabeled Ligand rate constant

$Q_{\min} \rightarrow$Minimum FSH level

$Q_{\max} \rightarrow$Maximum FSH level

$R_{\min} \rightarrow$Minimum LH level

$R_{\max} \rightarrow$Maximum LH level

$\tau_{FSH} \rightarrow$Response delay for FSH out

$\tau_{LH} \rightarrow$Response delay for LH out

$E_{cG} \rightarrow$Hill function coefficient in GnRH production

$n_{GQ} \rightarrow$Hill function exponent in FSH inhibition

$G_{cL} \rightarrow$GnRH hill function coefficient in LH release

$\bar{t} \rightarrow$Incubation time

5.3 Mathematical model

Consider the hormone levels as quasi steady functions of non peptide ligands and ligands. The kinetics used to describe association (H^+) and dissociation (H^-) are taken to be proportional to normalized functions ($i.e$ $0 \le H^{+\backslash -} \ge 1$), defined as follow

$$H^+(x,x_c,n)=\frac{\left(\frac{x}{x_c}\right)^n}{1+\left(\frac{x^n}{x_c}\right)} \qquad H^-(x,x_c,n)=\frac{1}{1+\left(\frac{x^n}{x_c}\right)} \tag{5.1}$$

Where both functions are strictly monotone, with H^+=0 and H^-=1 at $x = 0$ and H^+=1 H^-=1 at $x \to \infty$

The constant $x_c$is the hill function coefficient, such that $H^{+\backslash -}(x,x_c,n)=\frac{1}{2}$, and n is the hill function exponent that governs how sharp the function jumps from 0 to 1 or vice versa in the vicinity of $x = x_c$. Small integer exponents n (e.g n=1,n=2) are relatively straightforward to explain using the ideas from enzyme kinetics**[16,37,108]**, whilst larger values would suggest that the underlying processes being described by these kinetics are much more complex

$$\frac{dF}{dt}=(\alpha_f+\alpha_{fs}H^+(S,S_c,n_S))F-\left(\beta_f F^2\right)H^+(L,L_c,n_c) \tag{5.2}$$

$$\frac{dR}{dt} = \gamma_f H^+ \left(L, L_c, n_c\right) F^2 - \gamma_c R \qquad 5.3$$

$$\frac{dC}{dt} = \gamma_f R - \delta_c C \qquad 5.4$$

The variables F, R and C represent masses of uracil-1 (u1), uracil-2(u2) and uracil-3(u3), S and L in the Hills functions are the concentration levels of FSH and LH. The remaining parameters are subscripted and the subscription relates the parameter to the hormones **[11,89].**

The kinetics in equations (5.2) and (5.3) involves FSH and LH that are released by ligand constitutively and in response to GnRH levels, denoted by G, Taking FSH (Q) and LH(R) at quasi-steady levels. It can be written as

$$Q = \left(Q_{\min} + \left(Q_{\max} - Q_{\min}\right) H^+ \left(G\left(t - \tau_{FSH}\right); G_{cQ,} n_{GQ}\right)\right)\left(H^- \left(E, E_{cQ}, n_{EQ}\right)\right) \qquad 5.5$$

$$R = R_{\min} + \left(R_{\max} - R_{\min}\right) H^+ \left(G\left(t - \tau_{LH}\right); G_{cQ,} n_{GR}\right) \qquad 5.6$$

where τ_{FSH} and τ_{LH} are the lag time between ligand by GnRH and the release of FSH and LH.These formulations ensure that $Q \in \left(Q_{\min}, Q_{max}\right)$ and $R \in \left(R_{\min}, R_{max}\right)$ where the minimum values reflect background levels in the absence of GnRH; in humans, measured data indicates (Figure 5.2) that these hormones always present and constitutive production has been experimentally demonstrated in animals.

The overall effect of ligand is modeled as a quasi-steady, defined as

$$G(t)=\frac{1}{2T_S}\int_{T_S}^{T_S} G_{ligands}\left(t-\bar{t}\right)d\bar{t} \qquad 5.7$$

Where $G_{ligands}$ is the measured GnRH and T_s is a suitable time span to calculate.

The output of non-peptide antagonists on the GnRH **[20**] is written as

$$G=G_{\min}+\left(G_{\max}-G_{\min}\right)H^{+}\left(u_1,u_2,u_3\right)H^{-}\left(u_1,u_2,u_3\right) \qquad 5.8$$

Where G_{min} and G_{max} are the minimum and maximum levels of GnRH and so G∈ (G_{min}, G_{max}).

The current understanding of GnRH physiology comes from various studies of animals as well as human models by experimentally manipulating the hypothalamus and pituitary interactions, which permit validation on GnRH secretion and the subsequent LH release **[31, 81, 117].**

Numerical solution of the differential equations (5.2)-(5.4) are performed using Numerical Algorithms Group, which uses a backward differences formula to integrate a stiff system of first order differential equations over an interval with suitable initial conditions. This demonstrates that the relatively simple model can qualitatively reproduce many of the important biological observations regarding Ligand Association and Dissociation rate constant activities.

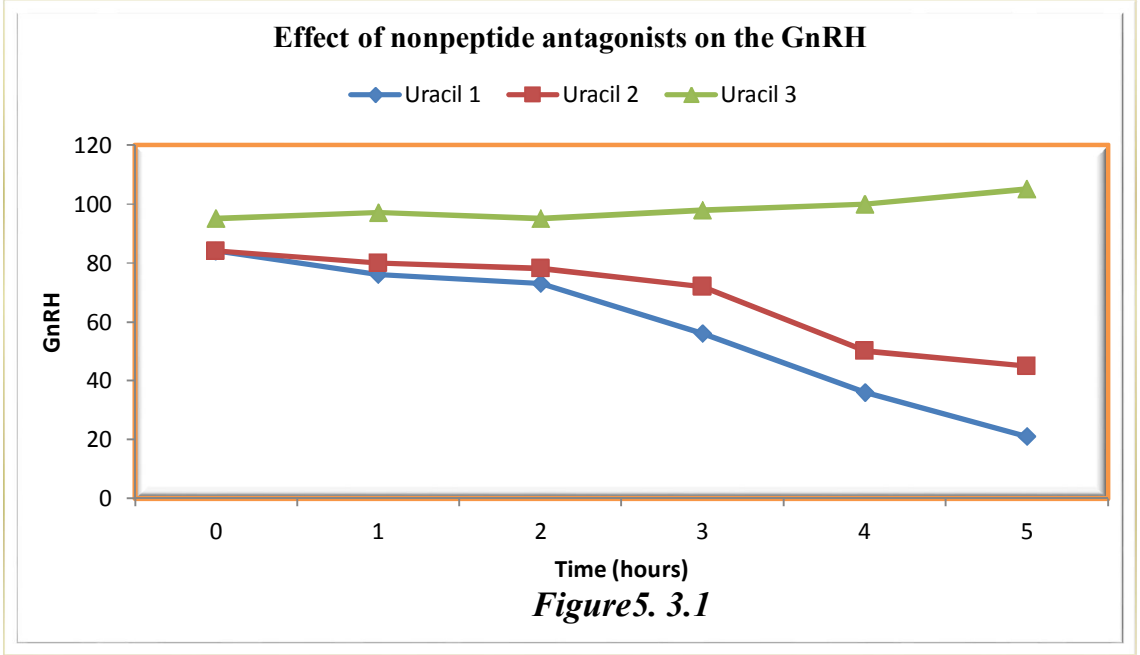

Figure5. 3.1

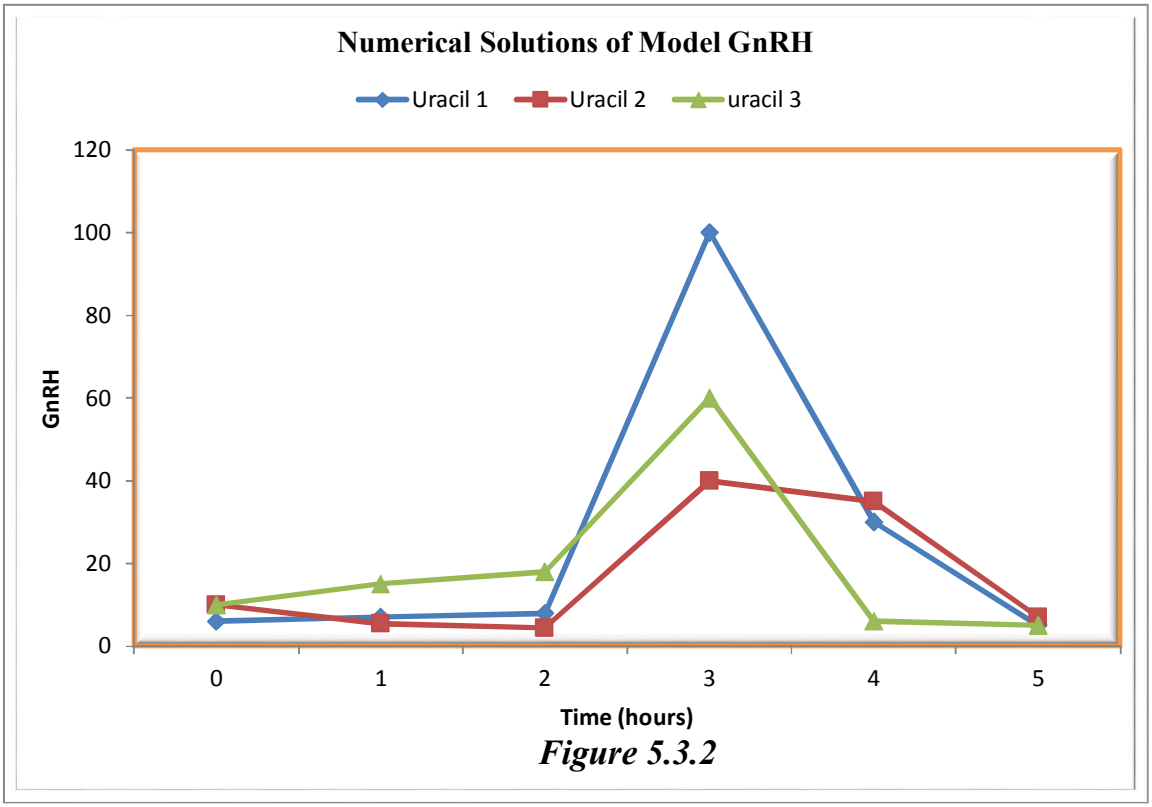

Figure 5.3.2

5.4 Conclusion

A large diversity of non-peptide ligands have been developed for the GnRH receptor, with the aim of developing orally available agents for the treatment of diseases associated with the reproductive non peptide antagonists. The SAR of these antagonists has typically been assessed using measurements of ligand affinity in ligand displacement assays or ligand potency in functional inhibition experiments.

The kinetics of non-peptide antagonist ligand interaction with the GnRH receptor is evaluated utilizing a novel ligand, and found a structure activity relationship defined by the dissociation rate of the ligand from the receptor. This difference of receptor ligand interaction was poorly resolved in standard experiments these findings could be useful in the further development of nonpeptide antagonists targeting the GnRH receptor. A Mathematical model describes the hormonal interaction of the human gonadotropin-releasing hormone (GnRH). The model is formulated on the bases of biologically relevant mechanisms and the model`s parameter have a clear biological interpretation, this model to include the role of GnRH receptors to simulate the effect of GnRH agonist treatment.