Delay Mechanism Involving a Drug Target Candidate G Protein Coupled Receptors in Signal Pathways

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Abstract: - G protein coupled receptors (GPCRs) constitute the largest family of cell membrane receptors which are subject to being targeted by an estimated 50% of current pharmaceuticals. Thus, better understanding of GPCRs and the signal transduction pathways they initiate will lead to new drug targets. Signal transduction is the process by which a cell recognizes and extracellular signal and converts that signal into an intracellular response. Subjected to transient stimuli, biological systems can exhibit early responses and/or late responses. In this study, we use mathematical modelling and analysis to study dynamical mechanisms of biological memory and delayed response to external stimuli. A delay model of signaling pathways involving G-proteins is analyzed to show that the model admits positive solutions and is uniformly persistent. Further, it is found that the delays $\tau_i$ in response to inhibition and $\tau_R$ in G protein mediated response to external stimuli of the receptors do not appear to impact on the persistent characteristics of this system.

Key-Words: - Delay differential equations; omega limit set; persistence; signal transduction; stability

1 Introduction
It is well recognized that G-protein-coupled receptors (GPCRs) are the largest family of cell membrane receptors [1]. The fact that an estimated 50% of current pharmaceuticals target GPCRs [2] indicates that further increases in our understanding of GPCRs and the signaling pathways they mediate will without doubt lead to new drug targets. Use of mathematical and computational models has played an increasingly important role in modern biology and pharmacology [1, 3, 4] and offers a powerful tool for examining GPCR pathways. Apart from allowing us to better understand hypothesized mechanisms, they can be used to execute virtual (in silico) experiments, interpret data, suggest new drug targets, motivate a design of experiments, and offer new or insightful explanations for observed phenomena [1].

Abnormalities of signal transduction pathways have been linked to the development of many serious disorders, such as cancer which derives from a cell that has lost the ability to respond normally to controls from outside, or inside, the cell. Signal transduction is the process by which information from an extracellular signal is transmitted from the plasma membrane into the cell and along an intracellular chain of signaling molecules to stimulate a cellular response [5].

The signal transduction pathway is a three step process; reception, transduction, and the response step. Reception is the target cell's detection of a signal transmitted from cell's surrounding environment. A chemical signal is detected when it binds to a receptor protein located at the cell's surface or inside the cell. Signal transduction converts the external stimuli into a form that can bring about a specific cellular response. In the third stage of cell signaling, the transduction process brings about a cellular response. This can be any of many different cellular activities, such as activation of a certain enzyme, rearrangement of the cytoskeleton, or activation of specific genes [6]. After a signal transduction pathway has been initiated and the information has been transduced to affect other cellular processes, the signaling processes must be terminated. Without such termination, cells lose their responsiveness to new signals. Signal processes that fail to properly terminate can lead to uncontrolled cell growth and the possibility of cancer [7]. In addition, we know of many situations where altered signaling pathways produce dramatic changes in cell survival, cell
proliferation, morphology, angiogenesis, longevity, or other properties that characterize cancer cells [8]. For this reason, better understanding of the signal transduction process has been a subject of intense investigation.

In the work of Rattanakul et al. [9], a model was proposed for the signal transduction pathway which involves G protein coupled receptors (GCPRs) consisting of a system of two differential equations governing the interaction between the inhibitor protein and the ligand-receptor complexes. Signal transduction across the plasma membrane is mediated by membrane receptor bound proteins which connect the genetically controlled biochemical reactions in the cytosol to the production of the second messenger, leading to desired intracellular responses.

According to Han et al. [10], memory is a ubiquitous phenomenon in biological systems, yet its impacts, and how to manipulate it at the subcellular level, remain poorly understood. Subjected to transient stimuli, biological systems can exhibit short early responses and/or prolonged late responses [6]. Although experimental evidence has provided some intuitive explanation at the basic molecular level, it does little to clarify the important dynamics that could lead us to discover possible therapeutic strategies in the setting of human diseases. In such attempts, mathematical modelling can go a long way in illuminating the underlying dynamic intricacies which may not be attained in experimental executions alone.

In this work, two significant time delays have been incorporated in the system. One is the delay \( \tau_I \) in the response of the ligand-receptor complexes (\( R(t) \)) to the action of the inhibitor protein, while the other is the delay \( \tau_R \) in the response of the inhibitor protein (\( I(t) \)) to the changes in the desity of \( R(t) \).

Based on earlier investigations and modeling efforts of Giang et al. [11] and Palumbo et al. [12], we study a mathematical model for the signal transduction process consisting of delay-differential equations modified from the model studied by Rattanakul et al. [9]. The model is then analyzed by using the \( \omega \)-limit set of a positive solution and constructing a full time solution to show that the model is persistent under certain conditions, in which case the levels of the inhibitors and ligand-receptor complex are bounded above and below by positive constants. Moreover, under certain conditions, oscillation about the respective basal levels, which is of clinical interest for control purposes, may be observed or else the system converges to a positive steady state.

2 The Reference Model

In this paper, we consider the system of two delay differential equations which governs the interaction between the ligand-receptor complexes \( R(t) \) and the inhibitor protein \( I(t) \) as follows:

\[
\frac{dR}{dt} = -a_R R - \frac{b_R}{b_I + R^2} \left( \frac{b_I R^2}{(b_I R + I(t-\tau_I))^2} + a_4 \right) \quad (1)
\]

\[
\frac{dI}{dt} = -a_I I + a_R R(t-\tau_R) \quad (2)
\]

where the first term on the right of (1) and (2) are the removal rates of the corresponding state variables, the second term on the right of (1) is the rate that \( R \) is internalized through the cell membrane, the third term accounts for the amplification effect on the production of \( R \) due to the secretion of the secondary hormone or signal with a delay \( \tau_I \), and \( a_4 \) is the zero order production rate of \( R \). The second term on the right of (2), on the other hand, is the production rate of inhibiting protein \( I \) in response of the increase in \( R \) at the time \( \tau_R \) see earlier. We refer the readers to the work of Rattanakul et al. [9] for more detail of the above model derivation. We first show that the model system (1)–(2) has a positive solution.

**Theorem 1** System (1)–(2) admits positive solution for any positive initial condition provided that \( R(t)>0 \) on the initial interval \([-\tau_R,0] \) and \( I(t)>0 \) on the initial interval \([-\tau_I,0] \).

**Proof.** Let \( R(t)>0 \) over an initial interval \([-\tau_R,0] \). According to the continuity of the solution of a differential equation, \( R(t) \) would become non-negative if there existed a \( t_0 > 0 \) such that

\[
R(t_0) = 0
\]

and

\[
R(t) > 0 \quad \text{for any} \quad 0 \leq t < t_0.
\]

Then, necessarily, \( \frac{dR}{dt} \bigg|_{t=t_0} \leq 0 \), which is a contradiction because

\[
\frac{dR}{dt} \bigg|_{t=t_0} = -a_R R(t_0) - \frac{b_R}{b_I + R(t_0)^2} \left( \frac{b_I R(t_0)^2}{(b_I R(t_0) + I(t_0-\tau_I))^2} + a_4 \right)
\]

\[
= a_4 > 0.
\]

This proof that, if \( R(t)>0 \) over \([-\tau_R,0] \) then \( R(t) \) never vanishes and is positive for all \( t \geq -\tau_R \).
Similarly, it can be proven that, if \( I(t) > 0 \) over an initial interval \([0, \tau]\), also \( I(t) \) never vanishes and is positive for all later time. If there existed a \( t_0 > 0 \) such that
\[
I(t_0) = 0
\]
and
\[
I(t) > 0 \text{ for any } 0 \leq t < t_0.
\]
Then, necessarily, \( \frac{dI}{dt} \bigg|_{t=t_0} \leq 0 \), which is a contradiction because
\[
\frac{dI}{dt} \bigg|_{t=t_0} = -a_2 I(t_0) + a_4 R(t_0 - \tau) = a_4 R(t_0 - \tau) > 0.
\]

### 3 The Uniform Persistence

This section investigates some properties involving the solution of (1)–(2) and the equilibrium point \((b, I_b)\) which, by definition, satisfies the following system:

\[
a_R b_R + \frac{b_R}{b_2 + R_2} - \frac{b_R}{(b_R^2 + I_b)^2} = a_5
\]

\[
I_b = \frac{a_3}{a_5} R_b
\]

In what follows, we let

\[
R_m = \liminf_{t \to +\infty} R(t), \quad R_M = \limsup_{t \to +\infty} R(t),
\]

\[
I_m = \liminf_{t \to +\infty} I(t), \quad I_M = \limsup_{t \to +\infty} I(t).
\]

**Theorem 2** System (1) – (2) is persistent.

**Proof.** Recall that a model is persistent if there exists a pair of positive real numbers \((m, M)\) such that there exists a \( T \) such that

\[
0 < m < X(t) < M < +\infty, \quad \text{for all } t \geq T
\]

for each component \( X(t) \) of the state vector.

The proof is achieved by proving the following four statements:

1) \( I_m < +\infty \), 2) \( R_m > 0 \), 3) \( I_M > 0 \), 4) \( R_M < +\infty \).

**Step 1.** In order to show the boundedness of the evolution of the inhibitor, assume that \( I_M = +\infty \), which means, due to continuity, that there is a time sequence \( \{t_n\} \subset [0, +\infty) \) such that

\[
\lim_{n \to +\infty} t_n = +\infty,
\]

and

\[
\lim I(t_n) = +\infty,
\]

with

\[
\frac{dI}{dt} \bigg|_{t=t_n} \geq 0.
\]

However,

\[
\frac{dI}{dt} \bigg|_{t=t_n} = -a_2 I(t_n) + a_4 R(t_n - \tau) \to -\infty,
\]

which is a contradiction, so that \( I_M < +\infty \).

**Step 2.** Suppose \( R_m < +\infty \) (otherwise \( R_m > 0 \) is trivially verified). Due to continuity, there exists a time sequence \( \{t_n\} \subset [0, +\infty) \) such that

\[
\lim_{n \to +\infty} t_n = +\infty,
\]

and

\[
\lim R(t_n) = R_m,
\]

with

\[
\frac{dR}{dt} \bigg|_{t=t_n} = 0.
\]

This means that:

\[
0 = \lim_{n \to +\infty} \left( -a_2 R(t_n) - \frac{b_R R(t_n)}{b_2 + R_2} \right. + \frac{b_R^2 (t_n)}{(b_R R(t_n) + I(t_n - \tau))} + a_4 \bigg)
\]

\[
= -a_2 R_m - \frac{b_R R_m}{b_2 + R_m^2} + \frac{b_R^2 I_m}{(b_R R_m + I_M^2)} + a_4
\]

\[
\geq -a_4 R_m - \frac{b_R R_m}{b_2 + R_m^2} + \frac{b_R^2 I_m}{(b_R R_m + I_M^2)} + a_4.
\]

Therefore, we have

\[
0 < a_4 \leq a_4 R_m + \frac{b_R R_m}{b_2 + R_m^2} - \frac{b_R^2 I_m}{(b_R R_m + I_M^2)}.
\]

According to this inequality, we must have \( R_m > 0 \), or otherwise we would have \( a_4 \leq 0 \).

**Step 3.** From Step 1, it follows that \( I_m \leq I_M < +\infty \). Due to continuity, there exists a time sequence \( \{t_n\} \subset [0, +\infty) \) such that

\[
\lim_{n \to +\infty} t_n = +\infty,
\]

and

\[
\lim I(t_n) = I_m,
\]

with

\[
\frac{dI}{dt} \bigg|_{t=t_n} = 0.
\]
This means that:
\[
0 = \lim_{n \to \infty} \left( -a_1 I_m(t_n) + a_1 R(t_n - \tau_R) \right) \\
= -a_1 I_m + a_1 R(t_n - \tau_R) \\
\geq -a_2 I_m + a_2 R_m.
\]

Then, we have \( a_2 R_m \leq a_1 I_m \). Therefore, \( I_m > 0 \), or otherwise we would have \( a_2 \leq 0 \).

**Step 4.** In order to show the boundedness of the evolution of the inhibitor, assume that \( R_M = +\infty \), which means, due to continuity, that there is a time sequence \( \{ t_n \} \subset [0, +\infty) \) such that
\[
\lim_{n \to \infty} t_n = +\infty,
\]
and
\[
\lim_{n \to \infty} R(t_n) = +\infty,
\]
with
\[
\frac{dR}{dt}\bigg|_{t=t_n} \geq 0.
\]

However,
\[
\frac{dR}{dt}\bigg|_{t=t_n} = -a_1 R(t_n) - \frac{b_1 R(t_n)}{b_2 + R(t_n)} + a_4 \to -\infty,
\]
which is a contradiction, and therefore \( R_M < +\infty \).

**Remark 3** As a consequence of Theorems 1 and 2, system (1) – (2) admits positive bounded solutions for any initial condition.

**Remark 4** Under the assumptions of Theorem 2, uniform persistence of the system (1)-(2) physically represents the fact that perpetual response to external stimuli, such as drug treatments, and inhibiting agents will be at work in a healthy subject.

**Theorem 5** Let \((R, I)\) be a bounded positive solution of (1)–(2). Then,
\[
I_m \leq I_b \leq I_M, \\
R_m \leq R_b \leq R_M.
\]

**Proof.** By using the \( \omega \)-limit set of the persistent solution \((R, I)\), we can construct a full time solution \((\mathcal{R}, I)\) such that
\[
I_M = I(0) = \max_{t \in \mathbb{R}} I(t), \\
I_m = \min_{t \in \mathbb{R}} I(t),
\]
\[
R_m \leq \mathcal{R}(t) \leq R_M, \quad \text{for all } t \in \mathbb{R}.
\]

The readers are referred to the works of Giang et al. [6], and Palumbo et al. [7], for more detail on full time solutions and their applications. It follows that \( I(0) = 0 \), and consequently,
\[
I_M = I(0) = \frac{a_1}{a_2} \mathcal{R}(-\tau_R).
\]

Below, it is proven that \( \mathcal{R}(-\tau_R) \geq R_b \), by showing that the assumption of \( \mathcal{R}(-\tau_R) < R_b \) leads to a contradiction. Indeed, if \( \mathcal{R}(-\tau_R) < R_b \), then \( R_m < R_b \), and by (4), we have
\[
I_M = \frac{a_1}{a_2} \mathcal{R}(-\tau_R) < \frac{a_1}{a_2} R_b = I_b,
\]
that is, \( I_M < I_b \). Again by using the \( \omega \)-limit set of the persistent solution \((R, I)\), we can construct a full time solution \((R, I)\) such that
\[
R_m = R(0) = \min_{t \in \mathbb{R}} R(t), \\
R_M \geq \max_{t \in \mathbb{R}} R(t),
\]
\[
I_m \leq I(t) \leq I_M, \quad \text{for all } t \in \mathbb{R}
\]
It follows that \( \dot{R}(0) = 0 \), and consequently,
\[
a_4 R_m + \frac{b_1 R_m}{b_2 + R_m^2} - \frac{b_2 R_m^2}{(b_1 R_m + I(-\tau_I))^2} = a_4
\]
However, in this case \( R_m < R_b \) and \( R_M < I_b \).

Therefore, we have
\[
a_4 = a_4 R_m + \frac{b_1 R_m}{b_2 + R_m^2} - \frac{b_2 R_m^2}{(b_1 R_m + I(-\tau_I))^2} \\
< a_4 R_m + \frac{b_1 R_m}{b_2 + R_m^2} - \frac{b_2 R_m^2}{(b_1 R_m + I(-\tau_I))^2} \\
< a_4 R_m + \frac{b_1 R_m}{b_2 + R_m^2} - \frac{b_2 R_m^2}{(b_1 R_m + I(-\tau_I))^2} = a_4
\]
which is a contradiction, so that \( \mathcal{R}(-\tau_R) \geq R_b \), and consequently, \( R_b \geq R_m \) and \( I_M \geq I_m \). Similarly, we may prove that \( R_m \leq R_b \) and \( I_m \leq I_b \).

**Remark 6** It is physically meaningful that the equilibrium is bounded in the range of all bounded positive solutions, and the densities of ligand-receptor complexes and the inhibiting protein in the transduction process should eventually adjust to some levels and remain steady when we are healthy.
It is easy to verify at this point that the following inequalities hold:
\[
a_I R_m \leq a_2 I_m \leq a_2 I_M \leq a_2 R_m, \tag{7}
\]
\[
a_I R_m + \frac{b_I R_m}{b_2 + R_m^2} - \frac{b_2 R_m^2}{(b_4 R_m + I_m)^2} \leq a_I \leq a_I R_m + \frac{b_I R_m}{b_2 + R_m^2} - \frac{b_2 R_m^2}{(b_4 R_m + I_m)^2} \tag{8}
\]

From the second inequality in (8), it follows that if \( I_m = I_b \), and by (3), we have \( R_m \geq R_b \), then \( R_m = R_b \). Now, the first inequality in (7) will give \( I_b \leq I_m \), so we can conclude that \( I_m = I_b \). That is \( \lim_{t \to \infty} I(t) = I_b \). In the same way, we have \( R_m = R_b \), or equivalently, \( \lim_{t \to \infty} R(t) = R_b \). This means that if the system solution does not oscillate about the equilibrium point \((R_b, I_b)\) then it must tend eventually toward the steady state. In other words, all solutions must oscillate about the steady state levels or else they converge to \((R_b, I_b)\) as time passes.

Remark 7 Every non-constant periodic solution of (1) and (2) must oscillate around the basal level \((R_b, I_b)\); otherwise, the inequalities (7) or (8) forces all strictly bounded positive solutions to converge to \((R_b, I_b)\).

4 Conclusion
It has been shown that the model of the signal transduction pathway consisting of two delay differential equations (1) and (2) is uniformly persistent. Moreover, it appears that the delays \( \tau_i \) and \( \tau_g \) do not impact on the persistent characteristics of this system. In fact, we found that a solution must oscillate about the steady state level \((R_b, I_b)\) unless it converges to the equilibrium point as time passes. When such oscillatory behavior may occurs and how the delays \( \tau_i \) and \( \tau_g \) effect the behavior of these oscillating solutions are subjects for future investigations.

The conclusions reached in this study are expected to bear important implications for experimental investigations to identify the mechanisms for biological memory and for the development of therapeutic strategies to modulate signaling network responses in the setting of human diseases

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