A Stochastic Differential Equation Model of the Signal Transduction Process Involving G-Protein Coupled Receptors

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Abstract: A stochastic model of the signal transduction process in the human body is proposed and investigated. Conversion of signals external to the cells into internal responses is mediated by a protein crucial to the control of the cells biochemical reactions to external stimuli, leading to the production of a secondary hormone or messenger, such as cAMP(Cyclic adenosine monophosphate). The G-protein coupled receptors (GPCRs) of external signals carried by messenger molecules are spread over the cell membrane surface. Experimental data on cAMP measurements exhibit seemingly random fluctuations as time progresses. Some researchers have suggested that the cell receptors receive the chemical signals in a stochastic fashion (Ueda and Shibata, 2007). The deterministic model discussed by Rattanakul et al. (2009) has thus been modified into a system of stochastic differential equations in order to take into account such stochastic nature. Estimations of the model parameters, together with related statistics, are found so that helpful understanding may be reached concerning the possible impacts of stochastic effects on the systems dynamic behavior leading to helpful conclusions.

Key–Words: G-protein coupled receptors, Parameter estimation, Signal transduction, Stochastic differential equation.

1 Introduction

A cell is the smallest unit of life which is functional in the living organism. The functions of a cell are growth, metabolisms, creation, protein synthesis, and so on. To perform its function, each cell has to interact with each other. One way this is done is through signaling. If the cell perceives and transmits the signal correctly, this can lead to proper development, repairing, and so on. However, if the cell responds to the signals incorrectly, this can cause many serious diseases [1].

The signaling process involves extracellular signaling molecules and cell-surface receptors. The extracellular signaling can transmit the signal over a short distance to stimulate cells that are close to the source or transmits the signal throughout the body. There are three types of signaling: autocrine signaling, paracrine signaling and endocrine signaling. The autocrine signaling is the process of signaling within the cell. This means that the cell uses the extracellular signaling to transmits the signal to its own receptors. For the paracrine signaling, the signal is sent from the cell to other cells that are close to the signal releasing cell. Lastly, the endocrine signaling is the process of signaling the cell that is far away from the signal releasing cell. The signal that is transmitted is actually hormones, which is secreted into the bloodstream and carried to the target cells [3].

The molecule that receives the signal is called a receptor. It is located on the surface of the cell. In addition, each cell has many receptors and each receptor responds to specific kinds of chemical signals. After the receptor receives the signal, it will relay the signal into a series of internal signaling molecules [6].

A cell signaling process can be divided into 3 stages: signal reception, signal transduction and cellular response [3]. The signal reception concerns the target cells detection of a signal transmitted from the cell's surrounding environment and the signal is detected when the ligand binds to a receptor. The signal transduction converts the external stimuli into a form which can bring about a specific cellular response. In the third stage of cell signaling, the transduced signal triggers a specific cellular response [3].

One type of receptors that comprises a large protein family of transmembrane receptors is called a G-protein-coupled receptor, which can only be found in eukaryotes. Their function is to detect molecules outside the cell and activate inside signal transduction pathways. It transmits various extracellular signals such as hormones, growth factors and neurotransmitters to the effectors such as adenylyl cyclase (AC) and phospholipase. Studies have indicated that a defect in the function of either the G-protein or GPCRs can lead to different kinds of human diseases [4, 17].

2 Transduction Process

Cells have a mechanism for detecting and responding to external signals. One of the more complex tactics for doing this concerns a three-stage G protein coupled enzyme cascade [17], a schematic diagram of which is in Figure 1.

In the first stage, the basal stage, the G protein which is constituted of 3 subunits: α, β and γ subunits, with GDP bound to the α - subunit, is activated by the receptor's interaction with a particular ligand.

In the second stage, the transduction stage, after the receptor has been activated and turned on the heterotrimeric G protein, by causing the G protein to replace GDP (guanosine diphosphate) by GTP (guanosine triphosphate), the GTP-bound α-subunit then dissociates from β and γ subunits and either or both regulates effector unit whose activity produces secondary messengers (cAMP).

The G protein subunit is transsient and it is terminated by the GTPase activity of the α - subunit. GTPase converts bound GTP to GDP then the protein becomes inactivated.
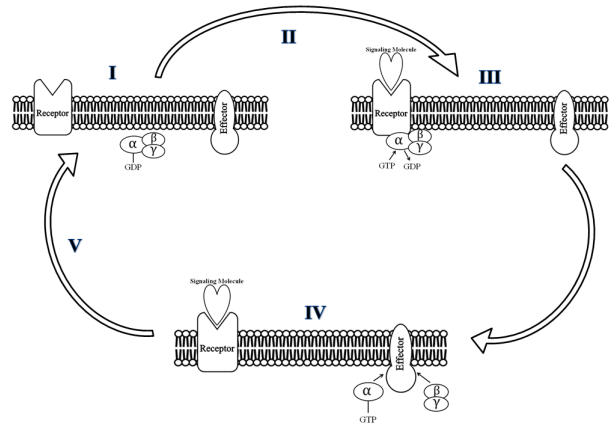

Figure 1: The G-protein GTPase cycle.

3 The Governing Equations

3.1 Deterministic model

We could think of the intracellular signal transduction in this way. Adenylyl cyclase (AC) occurs in two stages: active (R^*) and inactive (R). R is converted into R^* by a GDP bound α-subunit of G protein denoted by A and R^* is converted into R by GDP bound α-subunit whose amount is given by I. Moreover, A and I are activated by external signal membrane surface concentration (S). The activation of AC leads to the synthesis of cAMP (C) which regulates a downstream reaction to amplify the initial signal.
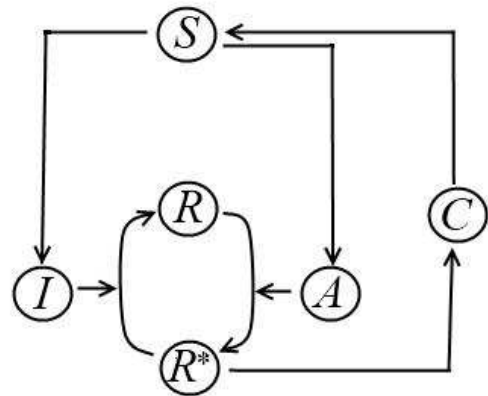

Figure 2: The reaction scheme

According to the several works [14, 15, 16]. We could consider the above dynamics in this way.

The equation of R^* can be written as

$$\frac{dR^*}{dt} = -k_{-r}IR^* + k_rAR \qquad (1)$$

where the first term on the right is the removal rate and the last term is the activation rate.

Assuming that the total amount of AC is constant denoted by R_T so that $R_T = R^* + R$, Equation (1) becomes

$$\frac{dR^*}{dt} = -\left(k_{-r}I + k_r A\right) R^* + k_r A R_T \qquad (2)$$

From the scheme seen in Figure 2, the dynamics of activator density (A) and inhibitor density (I) are described by the following equations

$$\frac{dA}{dt} = -k_{-a}A + k_a S \qquad (3)$$

and

$$\frac{dI}{dt} = -k_{-i}I + k_i A \qquad (4)$$

where the first terms on the right are the corresponding removal rates and the last terms are the corresponding rates of production.

The concentration of the second messenger or cAMP (C) which is synthesized as a result of enzyme R^* activation [13], satisfies the following equation

$$\frac{dC}{dt} = -k_{-c}C + k_{c1}[R^*]^2 + k_{c2} \qquad (5)$$

where the first term on the right is the removal rate and the last two terms represent the synthesis rate, k_{c2} being the zero order rate of production.

The dynamics of S follows the equation

$$\frac{dS}{dt} = -k_{-s}S - \frac{b_1 S}{b_2 + S} + k_s C \qquad (6)$$

where the first term on the right is the removal rate, the second term is the rate at which it is internalized through the cell membrane and the last term represents the signal amplification due to the secondary hormone C.

As argued in [5, 8], we may assume that the dynamics of R^*, A and C are relatively fast compared to the dynamics of I and S. Then, the values of R^*, A and C equilibrate quickly to

$$R^* = \frac{k_r A R_T}{k_{-r}I + k_r A} \qquad (7)$$

$$A = \frac{k_s}{k_a}S \qquad (8)$$

$$C = \frac{k_{c1}}{k_{-c}}[R^*]^2 + \frac{k_{c2}}{k_{-c}}. \qquad (9)$$

Substituting (8) in (4), we obtain

$$\frac{dI}{dt} = -a_1 I + a_2 S. \qquad (10)$$

where $a_1 = k_{-i}$ and $a_2 = \dfrac{k_a k_{-i}}{k_{-a}}$.

Substituting (7), (8), (9) in (6), we have

$$\frac{dS}{dt} = -a_3 S - \frac{b_1 S}{b_2 + S} + \frac{a_4 S^2}{(a_5 S + I)^2} + a_6 \qquad (11)$$

where $a_3 = k_{-s}$, $a_4 = \dfrac{k_{c1}k_s}{k_{-c}}\left(\dfrac{k_a k_r}{k_{-a}k_{-r}}R_T\right)^2$,

$$a_5 = \frac{k_a k_r}{k_{-a}k_{-r}} \quad\text{and}\quad a_6 = \frac{k_{c2}k_s}{k_{-c}}.$$

3.2 Formulation of the gradient-sensing SDEs model

Following the earlier works [2, 10, 11, 18], we could think about the above system (10)-(11) in this way.

Substituting Equation (7) in (9), one obtains

$$C = k\left[\frac{a_4 S^2}{(a_5 S + I)^2} + a_6\right] \qquad (12)$$

where $k = \dfrac{1}{k_s}$.

Then, we can write (11) as

$$\frac{dS}{dt} = -a_3 S - \frac{b_1 S}{b_2 + S} + \frac{C}{k}, \quad S(0) = S_0 \qquad (13)$$

when C is considered to be erratic.

We hypothesize that C is perturbed by a Gaussian white noise ξ,

$$C \to C + \tilde{\sigma}\xi$$

where $\tilde{\sigma}$ is a positive unknown parameter representing the noise intensity factor.

Then, we obtain

$$\frac{dS}{dt} = -a_3 S - \frac{b_1 S}{b_2 + S} + \frac{C + \tilde{\sigma}\xi}{k} \qquad (14)$$

Substituting $\tilde{\sigma}/k$ with σ,

$$dS = \left(-a_3 S - \frac{b_1 S}{b_2 + S} + \frac{C}{k}\right) dt + \sigma dW \qquad (15)$$

or

$$dS = \left(-a_3 S - \frac{b_1 S}{b_2 + S} + \frac{a_4 S^2}{(a_5 S + I)^2} + a_6\right) dt$$
$$+\sigma dW \qquad (16)$$

where W represents a standard Brownian motion.

Therefore, we arrive at the model consisting of the following equations

$$\frac{dI}{dt} = -a_1 I + a_2 S, \qquad I(0) = I_0 \qquad (17)$$

$$dS = \left(-a_3 S - \frac{b_1 S}{b_2 + S} + \frac{a_4 S^2}{(a_5 S + I)^2} + a_6\right) dt$$
$$+\sigma dW, \qquad S(0) = S_0 \qquad (18)$$

$$C = k\left[\frac{a_4 S^2}{(a_5 S + I)^2} + a_6\right] \qquad (19)$$

4 Parameter Estimation

4.1 Experimental data

To determine the above system (17) - (19) gives a proper model for the transduction process, we measured intracellular cAMP by using Fisher rat thyroid cells stably expressing type II antidiuretic hormone receptors, FRT-V2R, cultured in F-12 modified Coon's medium (Sigma) supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO_2.

Every two weeks, the FRT-V2R cells were selected with medium containing 500 μg/ml Zeocin, 500 μg/ml Geneticin and 350 μg/ml hygromycin. Then, the FRT-V2R cells were plated in 24-well plates overnight. After obtaining 80% of confluence, the cells were washed three times with PBS and incubated with 100 nM dDAVP (Sigma-Aldrich), a selective V2R agonist.

The incubation time was varied from 5 seconds to 16 minutes. The reaction was terminated by lysis buffer. After the incubation, cell lysate was transferred to 96-well plates. Then, the intracellular cAMP measurement using cAMP Biotrak EIA system (Amersham, GE Healthcare). The measurement protocol follows manufacturer's instructions, and samples were determined at optical density 450 nm [15].

Lowry method (1951) [9] was used to determined the amount of intracellular cAMP expressed per unit amount of protein.

To estimate the parameter values, we consider that the unknown model parameters ($\theta = [a_1, a_2, a_3, a_4, a_5, a_6, b_1, b_2, \sigma]$) could be estimated given the 2 equations. We use Euler-Maruyama approximation and Maximum likelihood estimator [12] to estimate the parameters upon the measured experimental data described above.

4.2 Euler-Maruyama approximation

Next, we consider the Itô SDE [7]

$$dX_t = f(X_t, \theta)dt + g(X_t, \theta)dW_t, \quad (20)$$
$$X(0) = X_0$$

where W is an m - dimensional standard Wiener process and

$$f : \mathbb{R} \times \Theta \to \mathbb{R} \text{ and } g : \mathbb{R} \times \Theta \to \mathbb{R}^{1\times m}$$

are known functions depending on an unknown finite-dimensional parameter vector $\theta \in \Theta$.

Considering the the Itô SDE (20) on $[t_0, T]$, for a given discretization $t_0 < t_1 < \cdots < t_n < \cdots < t_N = T$ of $[t_0, T]$, an *Euler-Maruyama approximation* is a continuous time stochastic process satisfying the iterative scheme

$$Y_{n+1} = Y_n + h_n f(Y_n) + g(Y_n)\Delta W_n, \quad (21)$$

$$Y_0 = X_0, \ n = 0, 1, \ldots, N - 1$$

where

$$Y_n = Y_n(t_n), \ h_n = t_{n+1} - t_n \text{ is the } stepsize,$$

$$\Delta W_n = W(t_{n+1}) - W(t_n) \sim \mathcal{N}(0, h_n)$$

with $W(t_0) = 0$ and $\mathcal{N}$ is the normal distribution.

4.3 Maximum likelihood estimator

The maximum likelihood estimator (MLE) of θ can be calculated if the transition densities $p(x_t; x_s, \theta)$ of X are knows, $s < t$. The log-likelihood function of θ is given by

$$l_n(\theta) = \sum_{i=1}^{n} \log p(x_i, x_{i-1}, \theta) \qquad (22)$$

and the maximum likelihood estimator $\hat{\theta}$ can be found by maximizing (22) with respect to θ. Under mild regularity conditions, $\hat{\theta}$ is consistent, asymptotically normally distributed and asymptotically efficient as n tends to infinity.

4.4 Results

The estimated parameter values can be found as given in Table 1.

Table 1: Estimated model parameters.

parameter	estimated value
a_1	0.90817 ± 0.03345
a_2	0.29170 ± 0.01829
a_3	2.14885 ± 9.38365
a_4	0.06629 ± 0.28644
a_5	0.31687 ± 0.88463
a_6	0.07902 ± 0.24257
b_1	0.31918 ± 2.49458
b_2	0.11690 ± 0.30378
σ	0.00954 ± 0.00249

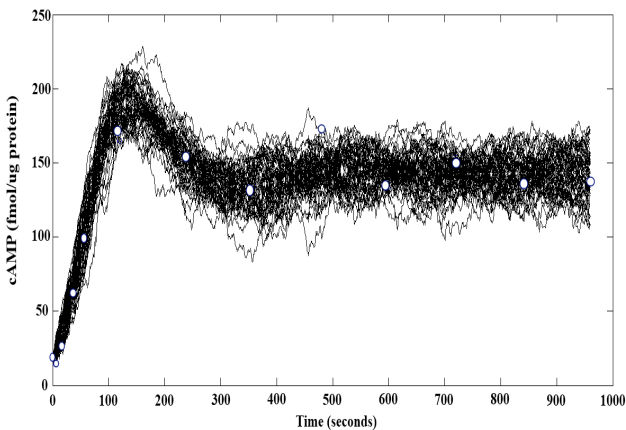Simulation results of the identified model are shown in Figure 3 and Figure 4.

Figure 3: Plots the numerical solution over 50 trajectories. The experimental measurements are shown as white dots.
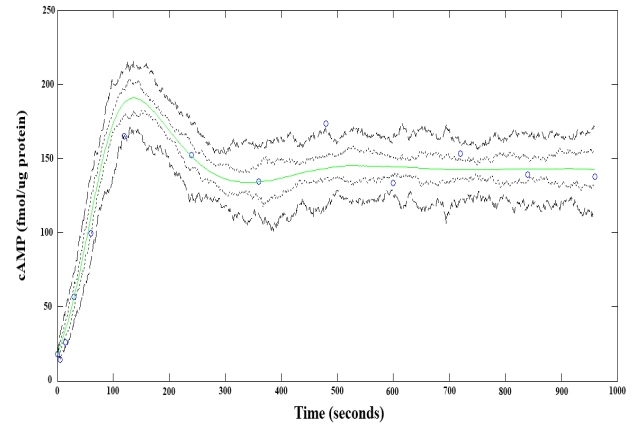

Figure 4: Plots the empirical mean (green solid line), 95% confident interval (dashed lines), Q_1 - Q_3 quartiles of the numerical solution (dotted lines) over 50 trajectories. The experimental measurements are shown as empty circles, while the empirical mean is shown here as a solid curve.

5 Conclusion

The aim of this paper was to estimate the parameters of the model for the experiment data in signal transduction involving G protein coupled receptors.

We developed a mathematical model by modifying the deterministic model proposed in [14] into stochastic model. Then, we estimated the parameter values by using the Euler-Maruyama Approximation and maximum likelihood estimators. Nine of the unknown parameter values could be estimated moderately well, given the limited data set of only 13 samples.

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