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Biophysical Chemistry 73 (1998) 31–52

Biophysical Chemistry

On the kinetic theory of the extracellular signal transduction in native cells: I. Hormone–receptor interaction

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Received 9 December 1997; received in revised form 9 February 1998; accepted 9 February 1998

Abstract

The hormone–receptor interaction is reconsidered and a minimal kinetic theory for the signal transduction in native cells is proposed. The basic equation for the time evolution of the concentration of the hormone–receptor complex is obtained. It is shown, that the hormone–receptor interaction is regulated by a cycle of G protein, i.e. kinetic parameters associated with the G protein cycle enter this equation. The existence of exponential and oscillatory regimes in kinetics of the G protein cycle, and hormone–receptor interactions are predicted. Experimental kinetic curves for agonist binding are computer-simulated, and numerical values for various kinetic and equilibrium parameters are obtained.

Keywords: Extracellular signal transduction system; Hormone–receptor interaction; Cycle of G protein; Complex dynamics; Computer simulation

1. Introduction

Over the last 4 decades multiple studies of hormone–receptor interactions have revealed the fact that a great variety of cell-surface receptors mediate their action by a transducing pathway, involving the activation of transducers, guanine nucleotide-binding proteins (G proteins) [1,2].

A modern view on the basic function of G proteins is that they serve as membrane-bound transducers of biochemically or biophysically coded information. Hormone–receptor interaction plays an important role in the process of transformation of an external signal into a cellular response [3]. This signal is recognized by the receptor on the surface of the cytoplasmic membrane and transmitted through G proteins to appropriate signal amplifiers, which synthesize second messengers. It has been recognized for a long time that G proteins regulate the opening and closing of the signalling pathways. It is also known that G proteins are widely distributed in various

0301-4622/98/$19.00
PII S0301-4622(98)00116-1

tissues, and the structures of individual proteins are highly conserved [4]. In order to transform the extracellular signal, a receptor must interact directly with the G protein. Hence, the cycle of G protein and hormone–receptor interaction cannot be considered separately. In other words, it is not possible to develop a kinetic theory, describing the time evolution of the experimentally observable parameter (in our case, the concentration of the hormone–receptor complex), without taking into account the important coupling of receptor with G protein [5].

There exists a family of G proteins as well as receptors, and the coupling of a G protein to the appropriate effector system can in general be very complicated. It is nearly impossible to take into account all microscopic details of the operation of say, only one signal transducing system within a single living cell. On the other hand, no matter how complex the diverse reactions involved in the transduction of the extracellular signal appear, the basic principle of the function of G proteins as molecular switches is universal [6]. The amplifiers (effectors) are presently the least understood in molecular–kinetic terms in comparison with G proteins and receptors. Nevertheless, it can be expected that different types of signal transduction systems form a universality class. It then becomes possible to construct a minimal kinetic theory, incorporating the most important steps into a complex mechanism.

In this work an attempt is made to quantitatively understand how the signal transduction system works. First, we start from the consideration of hormone–receptor interaction. The importance of studies of hormone–receptor interactions comes from the fact that in kinetic experiments on native cells the concentration of the hormone–receptor complex is an experimentally measurable quantity. Secondly, we take into account the conjugation of the receptor with G protein. It turns out that the kinetic parameters of the G protein cycle enter the kinetic equation for the concentration of the hormone–receptor complex. Once we have included the cycle of G protein into the description of the system, the agonist- and antagonist-induced receptor binding acquires new meaning.

The G protein cycle forms a complex system. We also study the dynamic stability of this system. It turns out that under specific conditions the kinetic structure of the G protein cycle loses its simplicity and the system shows a complex dynamic behavior. This complexity may drastically affect hormone–receptor interactions and lead to temporal dissipative structures that manifest themselves in the appearance of oscillatory regimes in the kinetics of agonist-induced receptor binding. The superposition of several oscillatory modes coming from several pools of receptors gives rise to more complicated dynamics, which may seem like temporal chaos.

The obtained expressions for the time evolution of the intracellular concentrations of GTP and GDP, as well as the concentration of the hormone–receptor complex, allow one to simulate the experimentally observed kinetic curves using standard computer programs, and to obtain the numerical values of various kinetic and equilibrium parameters of the signal transducing pathway. To probe the applicability of the derived equation for the time dependence of the total concentration of the agonist–receptor complex to the experimental data, we have analyzed the experimental kinetic curves for the binding of the δ-opioid receptors agonist [^{3}H]DADLE in suspensions of native neuroblastomaglioma cells NG108-15 [7,8]. The obtained kinetic parameters for the agonist-induced receptor binding are in agreement with the experimental data.

2. The naive picture of hormone–receptor interactions

We begin with a consideration of hormone–receptor interactions. The accepted kinetic model is

$$\mathrm{H} + \mathrm{R} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \mathrm{HR} \qquad \text{(A)}$$

Here H, R, HR denote hormone, receptor and hormone–receptor complex, respectively, and k_1, k_{-1} are kinetic constants for the association of hormone to receptor, as well as dissociation of hormone from receptor. The kinetic analysis of

scheme A in terms of the concentration of the hormone–receptor complex leads to a differential equation:

$$\frac{\mathrm{d}(B_1)}{\mathrm{d}t} = k_1(H_0)[(R_0) - (B_1)] - k_{-1}(B_1) \qquad (1)$$

Here (B_1) stands for binding and denotes the concentration of the hormone–receptor complex HR, and (R_0), (H_0) are the initial concentrations of receptor and hormone. Separation of variables and integration yields the expression for (B_1):

$$[B_1(t)] = \frac{k_1(R_0)(H_0)}{k_1(H_0) + k_{-1}} \times \{1 - \exp[-(k_1(H_0) + k_{-1})t]\} \qquad (2)$$

This expression is widely used to simulate the experimental kinetic curves of the hormone (both agonist and antagonist)-induced receptor binding. It correctly predicts the exponential form of experimental curves. One may expect the exponential growth at early times, and the saturation of receptor binding later. Nevertheless, it completely fails to fit other experimental observations. First, there is no possibility for the receptor to exist in its high and low affinity states. Second, the obtained expression does not distinguish between the action of agonist and antagonist. It is well known that kinetic curves for binding of agonist and antagonist are qualitatively different. Third, it is not clear how the hormone–receptor interaction is connected to the agonist-stimulated GTPase activity of the G protein. These considerations bring us to the understanding that we must extend a naive picture of hormone–receptor interaction by taking into account an important coupling of the receptor to the cycle of the G protein.

3. Signal transducing pathway

3.1. General considerations

Let us first consider the cycle of G protein. The importance of this will become obvious a bit later. The GTPase cycle of G proteins has been derived from many lines of experimental evidence (Fig. 1) [2,6]. The receptor (R) exists in high affinity (R_h) and low affinity (R_l) forms. Formation of the high affinity state of the receptor takes place when the former couples to the GDP-bound form of G protein (G·GDP) and forms the first ternary receptor–G protein–GDP complex (RG·GDP) [9,10]. The coupling of receptor to GTP-bound G protein, and the formation of the second ternary receptor–G protein–GTP complex (RG·GTP), correspond to the formation of the low affinity state of the receptor. The activation of the receptor by extracellular ligand (H), i.e. the formation of the first quaternary hormone-receptor–G protein–GDP complex (HRG·GDP), induces the release of GDP associated with the α-subunit of G protein (α), subsequent binding with GTP, and formation of the second quaternary hormone-receptor–G protein–GTP complex (HRG·GTP). This leads to the dissociation of the second quaternary hormone–receptor-G protein–GTP complex to the hormone–receptor complex (HR) and GTP-bound G protein (G·GTP). After the receptor has dissociated from GTP-bound G protein, the dissociation of the bound hormone from the receptor in its low affinity state occurs. GTP-bound G protein dissociates into a dimer formed by β- and γ-subunits of heterotrimeric G protein ($\beta\gamma$) and a complex of GTP with an α-subunit of G protein (α·GTP). This complex activates an amplifier. After hydrolysis of bound GTP into bound GDP has occurred (α·GTP → α·GDP), the α·GDP complex is released from an amplifier [2,11]. The recombination of the α·GDP complex with $\beta\gamma$-dimer terminates the cycle.

The first property which all known extracellular G protein-mediated signal transduction systems share is that the pathway acquires only one direction, i.e. hormonal activation of the receptor precedes the GTPase activity. The second property is that the activation of the receptor is governed by the appropriate hormone, i.e. the G protein cycle is regulated on the one hand, by the extracellular signal. On the other hand, the rate of GTP hydrolysis and subsequent dissociation from the α-subunit of G protein is determined by the period of time of GTPase activity of the G protein. In other words, the cycle of G protein has a probabilisti-

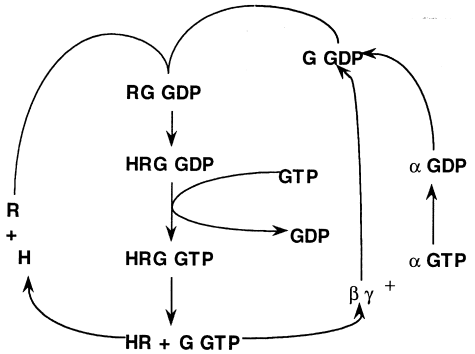


Fig. 1. The cycle of G protein. Abbreviations used: H, extracellular ligand (agonist, antagonist); R, free receptor; HR, the hormone–receptor complex; RG·GDP, ternary receptor–G protein–GDP complex corresponding to the active form of the receptor (R_h); G·GDP, the GDP-bound form of G protein; HRG·GDP, the first quaternary hormone–receptor–G protein–GDP complex; HRG·GTP, second quaternary hormone–receptor–G protein-GTP complex; G·GTP, GTP protein-bound G; $\beta\gamma$, β- and γ-subunits of G protein dimer; α·GTP, the complex of GTP with α-subunit of G protein; α·GDP, the complex of GDP with α-subunit of G protein.

cally-deterministic nature, i.e. the extent of external signal amplification is determined by the characteristic time during which the G protein possesses its GTPase activity and the time of interaction of the receptor with the messenger of information. These two properties are universal throughout the family of G proteins, i.e. the extracellular G protein-mediated signal transduction systems form a universality class.

All components that constitute the cycle are regenerated after one complete turn, except for GTP. In the long run we will have the excess of GDP over GTP that will bring about an unphysical increase in the concentration of the hormone–receptor complex due to the excess of high affinity receptors. On the contrary, experimental data indicate that receptor binding reaches the saturation plateau after the first 15–20 min have lapsed. It is unclear as to how the concentration of GTP returns to its physiological level after the system has been removed from exposure to the hormonal action. Therefore there should exist a GTP-regenerating system which restores the physiological level of the concentration of GTP. It is also clear, that this GTP-regenerating system and the cycle of G protein cannot be considered separately.

There exists a family of proteins that plays an important role in the maintaining of cellular concentration of GTP at the physiological level [12]. It was recently observed that along with cellular Nucleoside Diphosphate Kinase (c-NDP kinase), there exists membrane-associated Nucleoside Diphosphate Kinase (m-NDP kinase) which possesses chemical, kinetic and immunological properties close to those of c-NDP kinase (M_r = 18.300, $pI = 6.0$) [13]. It was also shown that m-NDP kinase is capable of transferring the terminal phosphate from ATP directly to GDP coupled with G protein [14]. This phosphorylation process can be schematically represented as follows:

$$\mathrm{ATP} + \mathrm{NDPkinase} \rightarrow \mathrm{ADP} + \mathrm{NDPkinase}\cdot p_i$$
$$\mathrm{NDPkinase}\cdot p_i + \mathrm{GDP} \rightarrow \mathrm{NDPkinase} + \mathrm{GTP}$$

If we take into consideration the GTP-regeneration by m-NDP kinase [15] and include it into the cycle of the G protein, the signal transducing pathway is closed and the law of conservation of matter is satisfied. The above considerations bring us to the extended view on the function of the G protein in mediating the extracellular signal transduction system (Fig. 2).

Now we turn to the construction of the minimal kinetic model for the external signal transducing pathway. We allow the transport of components of the signal transduction system from the place of high concentration to the place of low concentration. It is known that the hormone ($\sim 10^{-8}$–10^{-9} M) is present in excess over the receptor ($\sim 10^{-9}$–10^{-10} M), i.e. $H_0 = \mathrm{H} + \mathrm{HRG}\cdot\mathrm{GDP} + \mathrm{HRG}\cdot\mathrm{GTP} \approx \mathrm{H} \gg R_0$. It is also known that G protein is in excess (10^{-6} M) over the receptor, and that GDP and GTP are in excess (10^{-4}–10^{-5} M) over G protein [16]. This means that GTP and GDP exist in their free forms; 'free' receptor, i.e. receptor in absence of hormone, exists predominantly in its complex forms RG·GDP, RG·GTP (ternary complexes) and that the forms corresponding to the coupling of receptor to hormone are HRG·GDP, HRG·GTP. It also follows that G protein exists predominantly in forms of ternary (RG·GTP, RG·GDP) and quaternary (HRG·GTP, HRG·GDP) complexes,

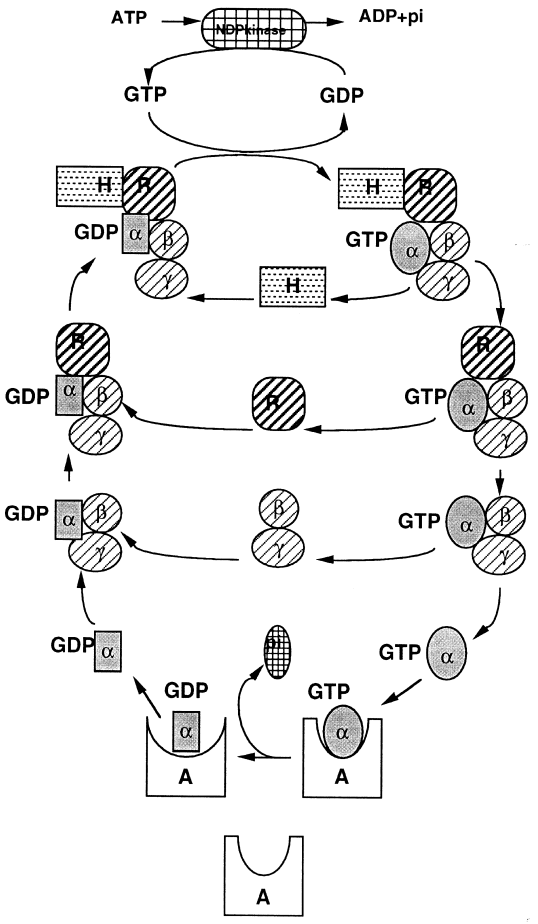


Fig. 2. The full cycle of G protein. Abbreviation used: p_i, inorganic phosphate; NDPkinase, membrane-associated NDP kinase. Other abbreviations used are the same as in Fig. 1. The cycle of G protein consists of three internal subcycles: the subcycle of the hormone H; the subcycle of the receptor R; and the subcycle of the $\beta\gamma$-dimer, and two external subcycles: the subcycle of the amplifier A; and the subcycle of m-NDP kinase.

$$G\cdot S_2 + R = RG\cdot S_2 \qquad K_0 = \frac{(RG\cdot S_2)}{(G\cdot S_2)\cdot(R)} \tag{1}$$

$$RG\cdot S_2 + S_1 = RG\cdot S_1 + S_2 \qquad K_1 = \frac{(RG\cdot S_1)\cdot(S_2)}{(RG\cdot S_2)\cdot(S_1)} \tag{2}$$

$$RG\cdot S_2 + H \xrightarrow{k_1} HRG\cdot S_2 \tag{3}$$

$$HRG\cdot S_2 + S_1 = HRG\cdot S_1 + S_2 \qquad K_2 = \frac{(HRG\cdot S_1)\cdot(S_2)}{(HRG\cdot S_2)\cdot(S_1)} \tag{4}$$

$$HRG\cdot S_1 \xrightarrow{k_{-1}} H + RG\cdot S_1 \tag{5}$$

$$RG\cdot S_1 \xrightarrow{k_2} R + G\cdot S_1 \tag{6}$$

$$G\cdot S_1 = G^*\cdot S_1 + \beta\gamma \qquad K_3 = \frac{(G^*\cdot S_1)\cdot(\beta\gamma)}{(G\cdot S_1)} \tag{7}$$

$$A + G^*\cdot S_1 = AG^*\cdot S_1 \qquad K_4 = \frac{(AG^*\cdot S_1)}{(G^*\cdot S_1)\cdot(A)} \tag{8}$$

$$AG^*\cdot S_1 \xrightarrow{k^*} G^* + S_2 + A \tag{9}$$

$$\beta\gamma + G^* = G \qquad K_5 = \frac{(G)}{(G^*)\cdot(\beta\gamma)} \tag{10}$$

$$G + S_2 = G\cdot S_2 \qquad K_6 = \frac{(G\cdot S_2)}{(G)\cdot(S_2)} \tag{11}$$

$$E_2 p_i + S_2 \xrightarrow{k_{cat}^{**}} E_2 + S_1 \tag{12}$$

Fig. 3. Kinetic model for the extracellular G protein-mediated signal transduction system. Abbreviations used: A, effector/amplifier; S_1, S_2 stand for GTP and GDP, respectively; G*, the α-subunit of G protein; $G^*\cdot S_1$ stands for $G^*\cdot$GTP — the complex of the active form of G protein (α-subunit of G protein) with GTP; $AG^*\cdot S_1$ stands for $AG^*\cdot$GTP — the complex of the amplifier with the α-subunit of G protein in its GTP-bound form; E_2 and $E_2 p_i$ stand for unbound and phosphate-bound forms of m-NDP kinase, respectively. Other abbreviations are the same as in Figs. 1 and 2.

as well as in its GTP- and GDP-bound forms ($G\cdot$GTP, $G\cdot$GDP).

Various reactions that constitute the G protein cycle, can be divided into reactions of association, dissociation, decomposition, substitution, and catalysis. We consider catalytic, hormone–receptor binding and dissociation reactions as well as the reaction of decomposition of the ternary complex of the receptor with G protein in its GTP coupled form, as irreversible steps (Fig. 3). The extent of external signal amplification is determined by the characteristic time during which G protein possesses its GTPase activity and the reverse of the value of the kinetic constant of association of receptor with hormone. These factors must determine a skeleton of the kinetic structure of the extracellular signal transduction system.

During steady-state hydrolysis of GTP into GDP, the great majority of G protein exists in the GDP-bound form, because k_{cat} for the conver-

sion of GTP into GDP exceeds k_{dis} for GDP (0.3 min^{-1}) by an order of magnitude [16]. This allows us to embed two consecutive processes into one limiting step $AG^* \cdot GTP \rightarrow G^* + GDP + A$ with the effective kinetic constant k^* (here A stands for amplifier, and G* stands for the α-subunit of G protein). The other key-step is the catalytic action of the hormone, $RG \cdot GDP + H \rightarrow HRG \cdot GDP$ with the kinetic constant for the binding of hormone k_1. It is believed that the bound hormone can also dissociate from the receptor with the kinetic constant of dissociation k_{-1}. We emphasize that this irreversible step takes place essentially after the equilibrium GDP/GTP-exchange process $HRG \cdot GDP + GTP = HRG \cdot GTP + GDP$ for the quaternary complex with the equilibrium constant K_2 has occurred. Therefore the hormone dissociation process is correctly represented as $HRG \cdot GTP \rightarrow H + RG \cdot GTP$. This is a crucial point in understanding the difference between the agonist and antagonist-induced receptor binding kinetics, as well as the absence of the cellular response upon the action of the antagonist. The ternary complex $RG \cdot GTP$ further decomposes into the receptor, and the GTP-bound form of G protein $G \cdot GTP$ with the kinetic constant of decomposition k_2: $RG \cdot GTP \rightarrow R + G \cdot GTP$. Since $RG \cdot GTP$ corresponds to the receptor in its low affinity state, this step must be fast in order to avoid the GDP/GTP-exchange (Fig. 3, step 2), and to ensure signal transduction. The complex $G \cdot GTP$ undergoes the reversible decomposition to the complex of the active form of G protein with GTP and $\beta\gamma$-dimer $G \cdot GTP = G^* \cdot GTP + \beta\gamma$ with the equilibrium constant K_3. The complex $G^* \cdot GTP$ further couples to the amplifier as $A + G^* \cdot GTP = AG^* \cdot GTP$ with the equilibrium constant K_4, and switches on a synthesis of the second messenger.

Now we take into account the regeneration of GTP by m-NDP kinase. The GDP-uptake during GDP-phosphorylation is an irreversible step [12]. The rate of phosphate transport is estimated to be 0.18 mM min^{-1}, so that it cannot be a rate-limiting step [13,14]. The phosphate-bound form of m-NDP kinase is obtained during the process of ATP-hydrolysis:

$$ATP + E_2 \underset{k_{-3}}{\overset{k_3}{=}} E_2 \cdot ATP \overset{k_{cat}}{\rightarrow} ADP + E_2 p_i$$

Here E_2 and $E_2 p_i$ stand for unbound and phosphate-bound forms of m-NDP kinase, respectively. k_3 and k_{-3} are the kinetic constants for association of ATP with m-NDP kinase and the dissociation of ATP from m-NDP kinase, respectively, and k_{cat} stands for the catalytic kinetic constant. In native cells, ATP is present in excess (10^{-3}–10^{-4} M) over m-NDP kinase ($\sim$ 10^{-5}–10^{-6} M), and the Michaelis constant for the ATP-hydrolysis process (K_m) is found to be equal to 0.4 mM [12]. It is therefore clear that m-NDP kinase exists predominantly in its phosphate-coupled form $E_2 p_i$, and we can consider the irreversible step of synthesis of GTP as $E_2 p_i + GDP \rightarrow E_2 + GTP$ with the kinetic constant k^{**}_{cat}. We also take into account the GDP/GTP-exchange $RG \cdot GDP + GTP = RG \cdot GTP + GDP$ for the ternary complexes with the equilibrium constant K_1. The resultant kinetic model for the function of the extracellular signal transduction system is shown in Fig. 3.

3.2. Hormone–receptor interaction reconsidered

Let us now go back to the problem of hormone–receptor interaction. In our extended view on the structure and function of the extracellular G protein-mediated signal transduction system the hormone–receptor interaction acquires a new meaning. We see that now it is absorbed into the extracellular signal transducing pathway, as the integral part.

Let us obtain the expression for the total concentration of the hormone–receptor complex. This complex exists in two forms, $HRG \cdot S_2$ and $HRG \cdot S_1$ (see Fig. 3). Therefore the total concentration of the hormone–receptor complex is a sum of concentrations of these forms, and the time evolution of the former is governed by the following expression (see Appendix A):

$$[B(t)] = (B_{ns})$$

$$+\frac{(R_0)e^{-k_{in}t}(1-e^{-k_1(H_0)(t+K_2Z(t)}}{1+K_2z(t)}$$

$$\times(1+K_2z(t)e^{-k_{-1}t}) \quad (3)$$

Here as before, (B) stands for binding and is the total concentration of the hormone–receptor complex. (B_{ns}) stands for non-specific binding and is linearly proportional to the concentration of hormone as $(B_{ns})=k_{ns}(H)$, where k_{ns} is the kinetic constant of non-specific binding. $z(t)$ is a function that reflects the ratio of the current concentrations of nucleotides (GTP/GDP-ratio):

$$z(t)=\frac{[S_1(t)]}{[S_2(t)]} \quad (4)$$

and $Z(t)'$ is a time derivative of $Z(t)$, such that

$$Z(t)'=z(t) \quad (5)$$

As is implied by Eq. (4), the concentrations of GTP and GDP are not constant over the time period of the hormonal action, and in general, functions of time.

It can be deduced from Eq. (3), that the extent of hormone–receptor interaction depends on the initial concentrations of hormone (H_0) and receptor (R_0), kinetic constants for association of hormone with receptor k_1 and dissociation of hormone from receptor k_{-1}, kinetic constant for the receptor inactivation k_{in}, current concentrations of GTP and GDP, and the equilibrium constant for the GDP/GTP-exchange, K_2.

The shape of kinetic curves for the binding of different ligands depends on values of the kinetic constants for binding k_1 and the dissociation k_{-1}. It is known that the kinetic constants for the binding of agonists to receptor are smaller than those of antagonists. In other words, antagonists possess a higher affinity for receptor than agonists. The difference between the action of the agonist and the antagonist is that the binding of the former to the receptor leads to the cellular response, while the binding of the latter does not. The cellular response can be generated only after the hormone has dissociated from the receptor. It follows that the action of agonists and antagonists must also be different in terms of kinetic constants for their dissociation from receptor. The value of the kinetic constant for the dissociation of antagonist from the receptor should be negligible in comparison with the value of the corresponding constant for agonist. Therefore we can formulate the difference between the action of agonist and antagonist in terms of the kinetic constants for binding and dissociation:

$$\begin{aligned}&k_1>0,k_{-1}>0\rightarrow\text{agonist}\\&\rightarrow\text{cellular response}\\&k_1>0,k_{-1}\approx 0\rightarrow\text{antagonist}\\&\rightarrow\text{no cellular response}\\&k_1(\text{agonist})<k_1(\text{antagonist})\end{aligned} \quad (6)$$

It is also instructive to introduce a relative logarithmic scale for the action of different hormones, based on the relative ratio of the kinetic constants for binding and dissociation:

$$r=-\log_{10}\frac{k_{-1}}{k_1} \quad (7)$$

Ligands with negative values of r correspond to antagonists, while the ligands with positive values of r correspond to agonists (Fig. 4).

It is possible to calculate the numerical value of the allosteric parameter (P) which reflects the ratio of the current concentrations of receptors in low and high affinity states, if the concentrations of the ternary complexes of the receptor and G protein in its GTP- and GDP-bound forms $(RG\cdot S_1, RG\cdot S_2)$ are known:

$$P=\frac{(R_l)}{(R_h)}=\frac{(RG\cdot S_1)}{(RG\cdot S_2)} \quad (8)$$

As is implied by the expression for the equilib-

Log10 [k1/k-1]

(...-6__-4__-2__0__+2__+4__+6...)
Antagonist Agonist

Fig. 4. The logarithmic scale for the ability of different ligands to produce the cellular response.

rium constant (see Fig. 3, step 2) $K_1 = (\mathrm{RG}\cdot S_1)\cdot(S_2)/(\mathrm{RG}\cdot S_1)\cdot(S_1) = P/z$, the allosteric parameter P is not a constant when z is a function of time, and is also a function of time, i.e. $P = f[S_1(t),S_2(t)]$.

In order to obtain an expression for the time evolution of the total concentration of the antagonist–receptor complex, we have to put $k_{-1} \approx 0$ in Eq. (3). We obtain:

$$[B(t)] = (B_{\mathrm{ns}}) + \frac{(R_0)(1 - e^{-k_1(H_0)(t+K_2Z(t)}}{1 + K_2 z(t)} \tag{9}$$

This equation does not contain the exponential prefactor $\exp[-k_{\mathrm{in}}t]$, taking care of the receptor inactivation, because there is no such process upon the action of antagonist. Using Eq. (3) and Eq. (5) we reconstruct kinetic curves for the agonist and antagonist-induced receptor binding (Fig. 5). Curve A for antagonist binding has a form of the exponential growth with saturation, which corresponds to the occupation of all receptors by hormones, while for agonist binding (curve B) this saturation decays in time. This decay is thought to correspond to a process of the receptors' inactivation. In fact, it is partially due to the process of dissociation of the agonist from the receptor.

It turns out that once we have incorporated the hormone–receptor interaction into the extracellular G protein-mediated signal transducing pathway, the agonist and antagonist-induced receptor binding is described by two different expressions (3 and 9). In order to simulate experimental data, we have to obtain the expressions for the time evolution of the concentrations of GTP and GDP $[S_1(t)$ and $S_2(t)]$ that enter Eq. (3) and Eq. (9) via functions $z(t)$ and $Z(t)$. This will be done in the next section.

3.3. Complex dynamics

Let us now go back to the minimal kinetic model for the extracellular signal transducing pathway. As is already mentioned, we should be interested in the dynamics of the components of the G protein cycle that enter equations, governing the dynamics of the hormone–receptor interaction, i.e. Eq. (3) and Eq. (9). Our goal is to obtain the equations governing the time evolution

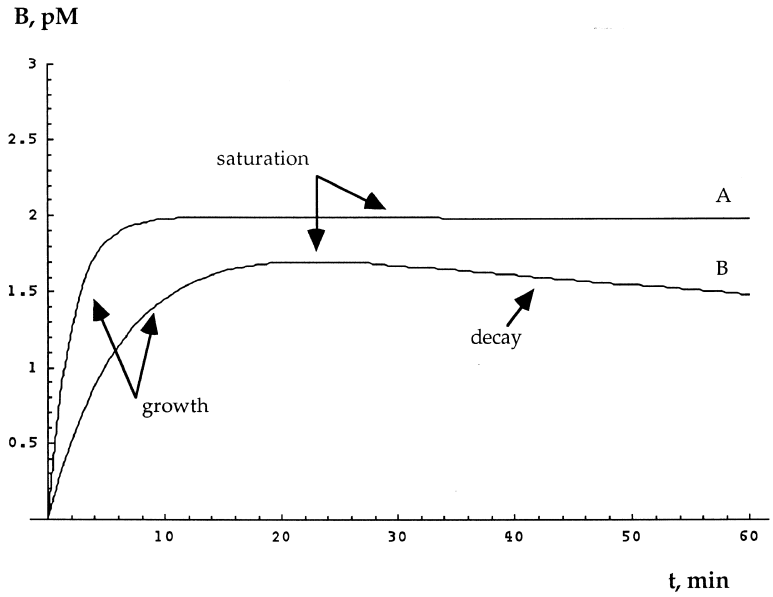


Fig. 5. Kinetics of the hormone–receptor interaction. Curves A and B represent the antagonist- and the agonist-induced receptor binding, respectively.

of the concentrations of GTP and GDP. These two components are present in excess over all other components of the G protein cycle and therefore the time evolution of the concentration of any component will be affected by the dynamics of GTP and GDP.

The equations for the concentrational change of GTP and GDP form a system of coupled linear differential equations (see Appendix B):

$$\frac{\mathrm{d}(S_1)}{\mathrm{d}t} = -k_t^1(S_1) + k_{\mathrm{cat}}^{**}(E_{20})(S_2) + k_t^1(S_{10})$$

$$\frac{\mathrm{d}(S_2)}{\mathrm{d}t} = -k^*K(S_1) - (k_{\mathrm{cat}}^{**}(E_{20}) + k_t^2)(S_2) + \left[k_t^2(S_{20}) + k^*K(S_{10})\right] \quad (10)$$

Here as before (S_1), (S_2) stand for the concentrations of GTP and GDP. (S_{10}), (S_{20}) stand for the initial concentrations of GTP and GDP, $(E_{20}) = (E_2 p_i)$ is m-NDP kinase in its phosphate-coupled form, constant K is the complex constant that includes the initial concentration of the amplifier (A_0) and the equilibrium constants K_3 and K_4:

$$K = \frac{K_3K_4(A_0)}{1 + K_3} \quad (11a)$$

For the meaning of the equilibrium constants K_3, K_4, and the kinetic constants k^*, k_{cat}^{**} we refer the reader to Section 3.1 and Fig. 3. We have also included linear terms $k_t^1 \cdot [(S_{10}) - (S_1)]$ and $k_t^2 \cdot [(S_{20}) - (S_2)]$, that correspond to transport of GTP and GDP with kinetic constants of transport k_t^1, k_t^2, respectively. The presence of these terms ensures that even if G protein and m-NDP kinase are spatially separated, the conversion of GTP into GDP and GDP into GTP does occur.

The molecules of GTP and GDP have a similar structure, and it should be expected that $k_t^1 \approx k_t^2$. Nevertheless, it is not always true. In general, sources of GTP and GDP may differ in their microenvironment (see discussion on the origin of pools of receptors below). The transport of GTP can be more difficult than transport of GDP, and vice versa. For example, m-NDP kinase (G protein) may be isolated by the intracellular compartmentalization from the rest of the signal transduction system. It follows that the ratio of the kinetic transport constants of GTP and GDP

$$k = \frac{k_t^1}{k_t^2} \quad (11b)$$

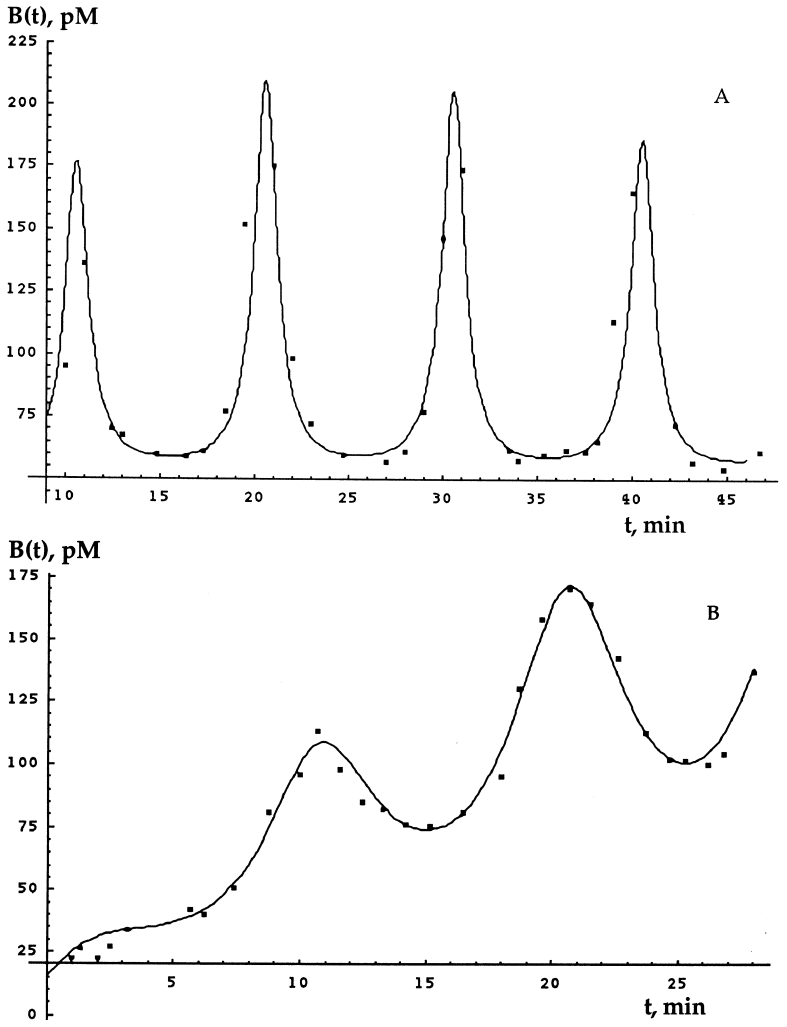
probes the difference in the geometry of the membrane-adjacent intracellular environment of G protein and m-NDP kinase. Clearly if the value of k is close to unity, the extracellular signal transduction system operates at its most efficient, and we call such a system *the perfect signal transducer*. In what follows we assume that $k \approx 1$.

The linear stability analysis (see Appendix C) shows that the system (10) is always stable with respect to small perturbations in the concentrations of GTP and GDP (see expressions C.12 and C.13).

The dynamics of the concentrational change of GTP and GDP affects the dynamics of the hormone–receptor interaction via functions $z(t)$ and $Z(t)$. Therefore the condition (C.9) also defines the regime for the dynamics of hormone–receptor interaction. The kinetic parameters entering the equations for the concentrational change in GTP and GDP are constants incorporating the kinetic and equilibrium parameters of various signal transducing steps, as well as the initial concentrations of different components of the G protein cycle. These parameters also enter the equation which governs the time evolution of the concentration of the hormone–receptor complex via functions $z(t)$ and $Z(t)$. Therefore in our extended view on the structure and function of G protein the hormone–receptor interaction becomes a structural part of the complex mechanism of operation of the extracellular signal transduction G protein-mediated system, as it should.

In a great majority of the experimental systems of native cells there exist different types of receptors of the same class [17]. For each type of receptors there might exist many different pools of receptors that can differ in their initial concentrations, as well as the kinetic and equilibrium

parameters of binding of the same hormone. This receptor inhomogeneity comes from the intracellular compartmentalization which divides a native cell into semiseparated compartments that are locally homogenous, i.e. the concentrations of individual components are constant within the same compartment, but may differ in the concentrations of same components from different compartments. Imagine now that different extracellular signal transduction systems within one cell are separated by intracellular compartmentalization. This separation leads to inhomogeneity in the initial concentrations of individual components of the signal transducing pathway and brings about inhomogeneity in equilibrium constants (see Fig. 3), as well as parameters entering the Eq. (3) and Eq. (9) that govern the dynamics of the hormone–receptor interaction, for example, the initial concentration of receptors (R_0), the equilibrium constant K_2, functions $z(t)$ and $Z(t)$ (see Eq. (C.4)), the expressions for $\lambda_{1,2}$ and the determinant D in Appendix C). In the case of oscillatory dynamics this will also give rise to inhomogeneity in the frequency of oscillations (see Eq. (C.11)). Therefore to obtain the correct expression for the total concentration of hormone–receptor complex, we have to sum the values of this parameter over different pools of



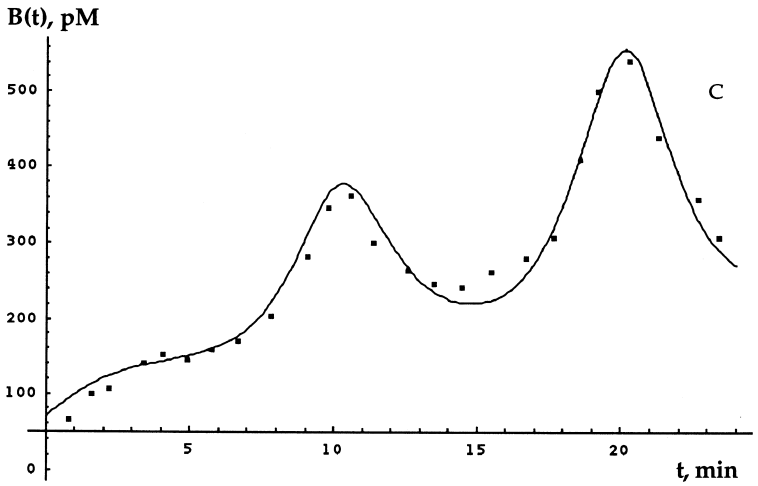


Fig. 6. The oscillatory kinetics for the receptor binding for the δ-opioid agonist [^{3}H]DADLE in the suspension of native neuroblastoma × glioma cells NG108-15. Experimental data points are computer simulated by the curves A–C.

receptors:

$$[B(t)] = \sum_{j=1}^{j=k} B_j(t) + k_{ns}(H) \tag{12}$$

We have also included a process of non-specific binding with the kinetic constant of non-specific binding k_{ns}. The summation $j = 1,2,...k$ runs over different pools. Each pool j of receptors contributes to the value of binding $B_j(t)$. The corrected expression for the agonist-induced receptor binding is

$$[B(t)] = k_{ns}(H) + \sum_{j=1}^{j=k} \frac{(R_{0j})e^{-k_{in,j}t}(1 - e^{-k_{1,j}(H_0)(t+K_{2,j}Z_j(t)}}{1 + K_{2,j}z_j(t)} \times \left(1 + K_{2,j}z_j(t)e^{-k_{-1,j}t}\right) \tag{13}$$

The corrected expression for the antagonist-induced receptor binding is

$$[B(t)] = k_{ns}(H) + \sum_{j=1}^{j=k} \frac{(R_{0j})(1 - e^{-k_{1,j}H_0)(t+K_{2,j}Z_j(t)})}{1 + K_{2,j}z_j(t)} \tag{14}$$

3.4. Discussion

The developed kinetic theory predicts the appearance of the exponential regime in the time evolution of the intracellular concentrations of GTP and GDP (see relation C.9). It can be shown that after the first 5–10 min of the experiment have elapsed, functions z and Z reach their constant value, i.e. the time evolution of the concentrations of GTP and GDP reaches its stationary point. This means that the kinetics of agonist and antagonist binding (Eq. (3) and Eq. (9)) become independent of the dynamics of the concentrational change of GTP and GDP, and the theoretically obtained curves have shapes of the experimentally observed kinetic dependences (see Fig. 5, curves B and A, and Fig. 7, curves A and B).

The developed kinetic theory also predicts the appearance of the oscillatory regime in the kinetics of the time dependence for the concentrations of guanine nucleotides (see relation C.9). It is worthy to mention, that the phenomenon of agonist-induced oscillations in various systems of native cells has already been observed for the following signal transduction steps:

1. Oscillations of GTP during the process of microtubules polymerization and depolymerization [18].

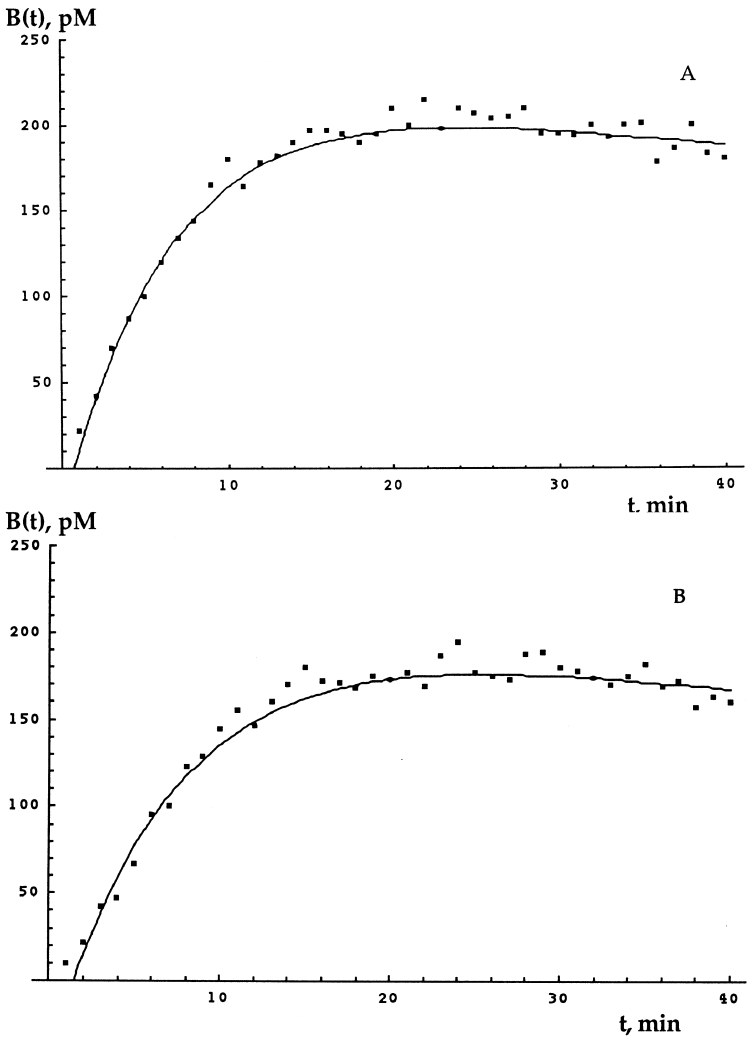


Fig. 7. The exponential kinetics for the receptor binding of the δ-opioid agonist [^{3}H]DADLE in the suspension of native neuroblastoma × glioma cells NG108-15. Experimental data points are computer simulated by the curves A and B.

2. Oscillations of cAMP in Dictyostelium discoideum [19].
3. Second messenger-oscillations of intracellular concentration of free calcium [20,21].
4. Steps activated by the second messengers, namely oscillations of calcium-dependent membrane potential on C6 cells [22].
5. Cellular response-oscillations of cAMP produced by Dictyostelium discoideum [23], respiratory burst [24], cellular shape change on human neutrophiles [25,26] and on Dictyostelium discoideum [23].

It is clear that the oscillatory behavior is an important property of the dynamically regulated extracellular signal transduction systems. The phenomenon of oscillations can give rise to the cooperative behavior of various subsystems which are involved in the function of the particular signal transduction system. In fact, oscillations arising at one point in the signal transducing pathway can be transmitted throughout the whole pathway by virtue of positive or negative feedback, including even steps preceding the point, where oscillations are generated.

From previous discussion, it follows that oscillations can be generated only by the agonist action. For the antagonist action the numerical value of the kinetic constant k_{-1} is equal (or close) to zero, and therefore there is no signal transduction. This means that the term $4k^*K$ in the determinant (D) (see Appendix C) is equal to zero and the latter becomes a positive quantity. It leads to the appearance of only the exponential regime in the time evolution of the concentrations of GTP and GDP, as well as the total concentration of the antagonist–receptor complex.

Both predicted exponential and oscillatory regimes for the hormone-induced receptor binding were experimentally observed within one type of receptors' system of native cells, namely for the δ-opioid agonist [^{3}H]DADLE (Tritium-labeled DADLE ((2-D-alanine-5-D-leucine)enkephalin)-induced receptor binding in suspensions of neuroblastoma × glioma cells NG108-15 [7,8,27]. For the conditions of cell cultivation and differentiation, as well as the experimental aspects of the detection of receptor binding we refer the interested reader to the original reference in Sukhomlin et al. [27].

The statistical analysis of the experimental data has shown that the discovered phenomenon of oscillations of the agonist-induced receptor binding is not an artifact and cannot be explained by experimental error. The statistical analysis has also shown that the experimentally observed kinetic curves for binding of the agonist have to be divided into three characteristic types: (1) exponential (Fig. 7, curves A and B); (2) oscillatory (Fig. 6, curves A–C); and (3) apparently chaotic (Fig. 8). In contrast, experimental kinetic curves for binding of the δ-opioid antagonist [^{3}H]DPN (Tritium-labeled diprenorphin) with receptors of NG108-15 cells are always exponential and have the shape of the curve A in Fig. 5.

Let us consider from now on only the agonist-induced receptor binding and go back to the results obtained in Section 3.2. The summation over different receptor pools in Eq. (13) implies the summation over receptors connected to various extracellular G protein-mediated signal transduction systems within limits of the individual cell, and further over the whole system of native cells. These signal transduction systems can be regulated by exponential and/or oscillatory regimes (modes) in the time evolution of concentrations of GTP and GDP. This leads to the situation, when within only one particular type of native cells an experimental kinetic curve is a superposition of exponential or oscillatory, as well

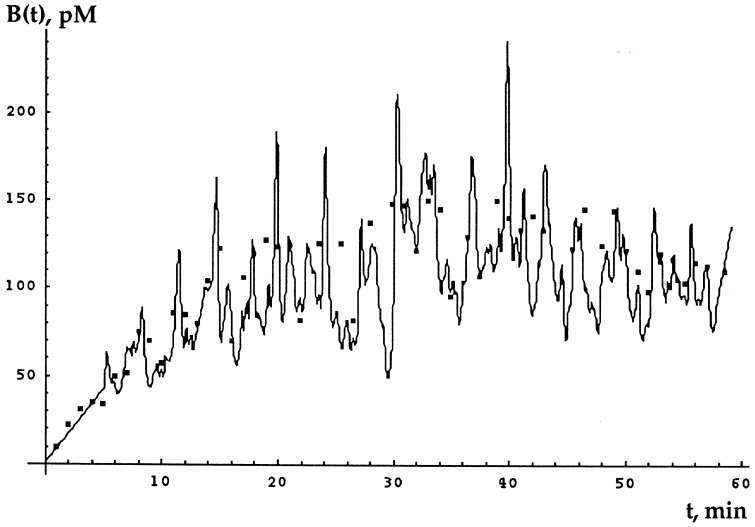


Fig. 8. Apparently chaotic kinetics for the receptor binding of the δ-opioid agonist [^{3}H]DADLE in the suspension of native neuroblastoma × glioma cells NG108-15. Experimental data points are computer simulated by the curve.

as both exponential and oscillatory modes coming from different pools of receptors. Clearly, the presence of only one exponential or oscillatory mode, corresponding to the existence of only one pool of receptors, or the superposition of only exponential modes, result in the overall simple exponential or oscillatory structure of the agonist binding kinetics (see Fig. 7, curves A and B; and Fig. 6, curves A–C). On the contrary, the superposition of several exponential and oscillatory, or only oscillatory modes, gives rise overall to a very complicated kinetic structure (see Fig. 8). One can observe, that the superposition of various oscillatory modes appears 'chaotic'. Indeed, it is tempting to consider this curve as the onset of temporal chaos. Nevertheless, Fourier analysis reveals the fact that this type of kinetics of agonist–receptor interaction remains deterministic, and is a superposition of several incoherent oscillatory modes, whose laws of evolution are known (see Section 4).

It is known, that δ-opioid ligands stimulate GTP-hydrolysis by virtue of binding to the δ-opioid receptors coupled to G protein in its GDP-bound form, with further activation of the GTPase-activity of the α-subunit of G protein [3,11]. This means that δ-opioid ligands bind to δ-opioid receptors and form the hormone–receptor complex. This primary messenger of the extracellular hormonal signal gets transmitted through G protein to adenylylcyclase (AC) which initiates the cellular response. The activation of this amplifier leads to the downregulation of the δ-opioid receptors activity (see Fig. 7, curves A and B). For these reasons, the system of δ-opioid receptors of NG108-15 cells can be taken as an appropriate experimental system to probe the applicability of the developed kinetic theory of the extracellular G protein-mediated signal transduction in native cells.

Various studies of the influence of different experimental conditions on receptor binding of native cells have revealed the fact that in the presence of reagents such as colchicine, and GTPγS (non-hydrolysable analog of GTP) that influence cell cytoskeleton or conjugation of receptors with G proteins, qualitatively changes the type of kinetics and increases the probability of the onset of the oscillatory regime.

These experimental observations can be explained within the framework of the developed theory. It is known that colchicine inhibits the process of polymerization of microtubules, and therefore reduces the degree of intracellular compartmentalization. The exposure of native cells to the action of colchicine leads to the situation when the α-subunit of G protein in its GTP-bound form couples to many molecules of the amplifier. This effectively increases the initial concentration of the amplifier (A_0), that GTPase sees. Now we go back to Section 3.3 and recall the relation C.9. According to this relation, an increase in the initial concentration of the amplifier elevates the probability of the onset of an oscillatory regime.

GTPγS stimulates the formation of the complex of m-NDP kinase with an α-subunit of G protein in its GTP-bound form [28,29]. If GTPγS is in excess over GTP then steps (9) and (12) (see Fig. 3) are suppressed. In the expression for $\lambda = -[k_t + \frac{1}{2}k^{**}_{cat}(E_{20})]$ (see Section 3.3) the second term becomes negligible, i.e. GTP is regenerated only by the diffusion process. This means that the absolute value of λ is decreased, and the amplitude of oscillations decays in time more slowly (see Eq. (C.4)). The overall effect is that oscillations live longer.

4. Computer simulations

To check the validity of the derived equation for the time evolution of the total concentration of the hormone–receptor complex, experimentally observed kinetic curves for the δ-opioid agonist [^{3}H]DADLE-induced receptor binding in suspensions of neuroblastomaglioma cells NG108-15 were simulated using standard computer program Mathematica 3.0 (Stephen Wolfram).

To simulate the experimental data, we use basic Eq. (3). The experimentally observed oscillatory kinetic curves were simulated in the approximation when the term $k^{**}_{\mathrm{cat}}(E_{20})$ in the determinant D (see Appendix C) is small in comparison to k^*K. Then the corresponding expressions for

the concentrations of GTP and GDP become:

$$(S_1) = (S_1^*) + e^{\lambda t}\left\{ \frac{(S_{10}) - (S_1^*)}{2\omega}\sqrt{\omega^2 + (Kk^*)^2} \sin\left[\omega t + \arctan\left(\frac{\omega}{k^*K} \right) \right] \right\}$$

$$(S_2) = (S_2^*) \quad (15)$$

This approximation is equivalent to the assumption that the oscillation in the concentration of GDP is of negligible amplitude. The result of the simulation of the experimental oscillatory kinetic curves is shown in Fig. 6, and the estimated numerical values for various kinetic and equilibrium parameters are accumulated in Table 1.

The experimentally observed exponential kinetic curves were simulated in the approximation when the term k^*K in the determinant D is small in comparison to $k_{cat}^{**}(E_{20})$. In this case, the corresponding expressions for the concentrations of GTP and GDP are:

$$(S_1) = (S_1^*) - [(S_{10}) - (S_1^*)]e^{\lambda_2 t}$$

$$(S_2) = (S_2^*) + [(S_{20}) - (S_2^*) - (S_{10}) + (S_1^*)]e^{\lambda_1 t} - [(S_{10}) - (S_1^*)]e^{\lambda_2 t} \quad (16)$$

The result of the simulation of the exponential kinetic curves is shown in Fig. 7, and the estimated numerical values for various kinetic and equilibrium parameters are accumulated in Table 1.

In the case of oscillatory kinetics, the estimated values of λ, and the frequency and the phase of oscillations can provide us with an insight into the numerical values of some important kinetic and equilibrium parameters of the G protein cycle. If we take the value of k^* to be equal to 0.3 min^{-1} [2,17], then we obtain $K = 27.6$, $k_t = 2.4 \times 10^{-2}$ min^{-1}, $k_{cat}^{**} = 4.8 \times 10^3$ min^{-1}, and $(E_{20}) = 10^{-5}$ M. It can be expected that the concentration of the effector (AC) is 10^{-5} M. If $K_3 \sim 1$, then we get $K_4 \sim 10^6$. In the case of the exponential kinetics, we can infer that $k_t \sim 10^{-3}$ min^{-1} and $(E_{20}) = 10^{-6}$ M.

Both for the oscillatory and the exponential kinetics the condition for the fixed points (S_1^*) (S_2^*) to be positive definite quantities, $(k_{cat}^{**}(E_{20}) > k_t)$ is satisfied. The estimated value of r for [^{3}H]DADLE (see the Eq. (7)) is found to be ~ 5 (Fig. 4).

To simulate the experimentally observed, apparently chaotic kinetic curves, we use Eq. (13). It turns out that for this type of kinetic dependences, the agonist-induced receptor binding is an additive quantity consisting of more than 30 components coming from various oscillatory modes with different values of frequencies varying between 0, 105 and 3.150 min^{-1}, and concentrations of receptor pools, varying between 0.5 and 50.0×10^{-11} M. All other kinetic and equilibrium parameters of receptors were kept identical. It turns out that the apparent chaos is the superposition of different incoherent modes. The result of the simulation is shown in Fig. 8, and the estimated numerical values for various kinetic and equilibrium parameters are accumulated in Table 1.

The obtained theoretical curve adequately reflects basic features of the 'chaotic' experimental kinetic curve. Nevertheless, it does not correlate well with all data points. The reason being, we do not have enough information in order to make a perfect fit. We do not know the precise number of different pools of receptors, nor do we know in which kinetic and equilibrium parameters they differ from one another. There are also computational problems arising from a complicated structure of this type of kinetic curves.

5. Concluding remarks

In this work we have made an attempt to quantitatively understand the operation of the extracellular G protein-mediated signal transduction system. It is shown that in order to explain the experimental observations, such as the existence of the receptor in its high and low affinity states, transmission of the extracellular signal, dynamics of hormone–receptor interaction should be closely connected to the operation of the G-protein cycle. We have also included membrane-associated NDP kinase (m-NDP kinase) into the G protein cycle. Clearly, the extracellular signal

Table 1
The numerical values obtained by the computer simulation of the experimental kinetic curves for the receptor binding of the δ-opioid agonist [^{3}H]DADLE in suspension of native neuroblastoma × glioma cells NG108-15

Kinetic/equilibrium parameter	Numerical value (curve A)	Numerical value (curve B)	Numerical value (curve C)
Oscillatory kinetics for agonist binding			
S_1^* (M)	1.4×10^{-5}	1.3×10^{-5}	1.1×10^{-5}
S_2^* (M)	9.3×10^{-5}	8.3×10^{-5}	7.3×10^{-5}
A (M)	5.2×10^{-5}	2.2×10^{-5}	2.6×10^{-5}
λ (min^{-1})	1.2×10^{-4}	1.1×10^{-4}	1.2×10^{-3}
k_1 (M^{-1} min^{-1})	2.1×10^{6}	3.1×10^{6}	3.5×10^{7}
k_{-1} (M^{-1} min^{-1})	33.2	20.6	0.28
k_{in} (min^{-1})	0.021	0.011	0.0092
K_2	2.1	1.7	1.6
R_0 (M)	1.0×10^{-9}	1.0×10^{-9}	7.0×10^{-10}
B_{ns} (M)	5.2×10^{-9}	1.3×10^{-9}	0.66×10^{-9}
ω (rad min^{-1})	0.63	0.63	0.63
f (rad)	4.34	4.34	4.64
Exponential kinetics for agonist binding			
S_1^* (M)	1.1×10^{-5}	1.0×10^{-5}	
S_2^* (M)	6.5×10^{-5}	6.7×10^{-5}	
S_{10} (M)	5.6×10^{-5}	3.5×10^{-5}	
S_{20} (M)	1.1×10^{-4}	0.9×10^{-4}	
λ_1 (min^{-1})	2.1×10^{-4}	2.0×10^{-3}	
λ_2 (min^{-1})	2.1×10^{-4}	2.0×10^{-3}	
k_1 (M^{-1} min^{-1})	7.1×10^{7}	6.4×10^{7}	
k_{-1} (M^{-1} min^{-1})	3.3	4.3	
k_{in} (min^{-1})	0.005	0.006	
K_2	7.2	7.2	
R_0 (M)	1.0×10^{-9}	1.3×10^{-9}	
B_{ns} (M)	$\sim10^{-13}$	$\sim10^{-13}$	
Chaotic kinetics for agonist binding			
S_1^* (M)	1.1×10^{-5}		
S_2^* (M)	8.0×10^{-5}		
A (M)	5.3×10^{-5}		
λ (min^{-1})	1.1×10^{-4}		
k_{-1} (M^{-1} min^{-1})	2.2×10^{7}		
k_{-1} (M^{-1} min^{-1})	20.2		
k_{in} (min^{-1})	0.021		
K_2	1.7		
B_{ns} (M)	$\sim10^{-13}$		
f (rad)	0.197		

The amplitude and the phase of oscillations are abbreviated as A and f.

transduction system cannot function if it does not involve the GTP-regenerating protein.

Based on the minimal kinetic model, we have proposed a kinetic theory for the extracellular signal transduction in a system of native cells. The obtained equations for agonist and antagonist-induced receptor binding are qualitatively different. This difference can be understood in the following way. The action of the antagonist does not lead to a cellular response. Indeed, this response cannot be initiated if the hormone does not dissociate from the receptor, and therefore, the GTPase activation process is impossible. GTPase does not couple to the amplifier, and there is no cellular response.

The synthesis of the molecules of the second

messenger down regulates the affinity of the receptor for the hormone. Hence, we should not observe the down regulation of the receptor upon the action of the antagonist. Therefore the equation for the binding of the antagonist should not include three parameters: kinetic constant for dissociation of antagonist from receptor k_{-1}; kinetic constant for inactivation of receptors k_{in}; and the equilibrium constant K, which incorporates different kinetic and equilibrium parameters of the G protein cycle.

We have not included the post-effector steps into our kinetic model. Notwithstanding the fact that post-effector steps are not well studied to the extent when any reasonable generalization is possible, it is known that these steps are responsible for the receptors inactivation (downregulation). This is why the kinetic constant of receptors inactivation does not appear in the final expression for the total concentration of the hormone–receptor complex as the intrinsic property of the extracellular signal transduction system. Instead, we have taken the receptor inactivation process into account in a rather phenomenological way. We have used the fact that this process is slow and have absorbed the factor $\exp[-k_{in}t]$ into the final expression for the time evolution of the total concentration of the agonist–receptor complex.

In order to determine if the ligand in question is agonist or antagonist, one needs to know the values of kinetic constants for binding k_1 and dissociation k_{-1} only. If these values are known, the information about cellular response becomes redundant. One can introduce the kinetic scale (parameter r) based on the logarithmic difference in orders of magnitude between k_1 and k_{-1}. This scale gives a quantitative measure for the ability of different ligands to produce a cellular response.

The kinetics of antagonist binding has a simple form, namely the exponential growth with saturation. The kinetics for the binding of the agonist can in general be very complicated. This complexity is associated with the dependence of the binding of the agonist on the ratio of current intracellular concentrations of guanine nucleotides (GTP/GDP-ratio). It is shown that generally GTP/GDP-ratio is not a constant, but rather a function of time.

The cycle of G protein is a complex system. The operation of such a system has many microscopic details. Nevertheless, the underlying mechanism is universal. The operation of the G protein cycle obeys two basic principles. The first one states that the kinetic mechanism of the extracellular signal transducing pathway acquires only one direction in a row of causality, that is, the agonist-induced receptor binding precedes the cellular response. The second principle states that the extracellular signal transduction system has the probabilistically-deterministic nature or built-in duality, i.e. the signal transduction is determined by both the time during which the hormone is bound to the receptor and characteristic time of GTPase activity.

Kinetic analysis of this system has demonstrated that under specific conditions the time evolution of the concentrations of GTP and GDP shows complex behavior. More specifically, depending on initial conditions (numerical values of various kinetic and equilibrium parameters, as well as the initial concentrations of the amplifier and m-NDP kinase), kinetics of the concentrational change for both GTP and GDP can be single-exponential, bi-exponential, and oscillatory. In the case of antagonist binding, the dynamics of the concentrational change of GTP and GDP has the exponential structure. In the case of agonist binding, the dynamics of the concentrational change of GTP and GDP can be exponential or oscillatory. The linear stability analysis has shown that the extracellular signal transduction system is always stable with respect to small perturbations in the intracellular concentrations of GTP and GDP.

The time evolution of the concentrations of guanine nucleotides affects the dynamics of hormone–receptor interaction. Our kinetic theory predicts the existence of exponential and oscillatory regimes in the dynamics of hormone–receptor interaction. The existence of pools of receptors leads to more complicated structure of the dynamics of hormone–receptor interaction. In the case of the superposition of several exponential and oscillatory, or only oscillatory modes, kinetic

curves for the agonist-induced receptor binding look apparently chaotic. This 'chaos' is not associated with the lack of predictability (deterministic chaos), and is a result of the superposition of incoherent oscillatory modes.

The δ-opioid receptors can be taken as an appropriate experimental system to probe the applicability of the developed kinetic theory of the extracellular signal transduction in native cells. Experimentally observed, both exponential and oscillatory kinetic curves for the δ-opioid agonist [^{3}H]DADLE binding in the suspension of native neuroblastoma × glioma cells NG108-15 were computer-simulated and numerical values for various kinetic and equilibrium parameters of receptor binding were obtained. Simulated curves correlate well with the experimental data set.

According to the prediction of our theory and the results of computer simulations, for the appearance of the oscillatory kinetics of agonist binding, the value of the catalytic kinetic constant for synthesis of GTP should exceed the value of the corresponding constant for GTP hydrolysis by at least one order of magnitude, and m-NDP kinase should be present in excess over G protein. The value of the kinetic constant for transport of GTP and GDP for oscillatory regime exceeds the value of the same constant for the exponential regime by one order of magnitude. This means, that the existence of oscillatory behavior in the agonist-induced receptor binding also depends on the geometry of the microenvironment of the signal transduction system.

Acknowledgements

Support of this work was provided by a grant from the US Department of Energy, DE-FG03-94ER14465. We thank Professor Ilya Prigogine for his support, and Professor Ilya Kurochkin under whose guidance present work was initiated. We would also like to thank Traci Petersen for her help in working on the manuscript, and Professor William Gardiner for his useful advises.

Appendix 1: Derivation of Eq. (3)

We start from differential equations for the concentrations of $HRG\cdot S_2$, $HRG\cdot S_1$. We introduce abbreviations. (B_0) and (B_1) stand for concentrations of $HRG\cdot S_2$ and $HRG\cdot S_1$, respectively. The equations are (steps 3 and 5 in Fig. 3):

$$\frac{d(B_0)}{dt} = k_1(RG\cdot S_2)(H)$$
$$\frac{d(B_1)}{dt} = -k_{-1}(B_1) \quad (A.1)$$

(B_0) and (B_1) are related via the expression for the equilibrium constant K_2 (Fig. 3, step 4)

$$K_2 = \frac{(B_1)(S_2)}{(B_0)(S_1)} = \frac{(B_1)}{(B_2)z} \quad (A.2)$$

where we have introduced the function $z = S_1/S_2$, which reflects the ratio of current concentrations of GTP and GDP. Concentrations of $RG\cdot S_2$ and $RG\cdot S_1$ are related via (Fig. 3, step 2)

$$K_1 = \frac{(RG\cdot S_1)(S_2)}{(RG\cdot S_2)(S_1)} = \frac{(RG\cdot S_1)}{(RG\cdot S_2)z} \quad (A.3)$$

The conservation equation for receptor is $(R_0) = (R) + (RG\cdot S_1) + (RG\cdot S_2) + (B_0) + (B_1)$. It follows (Section 3.1) that we can neglect the concentration of the receptor in its free form (R). Since the hormone is present in excess over the receptor, we always have $(H) = (H_0)$. Step 6 (Fig. 3) is fast, and we can neglect the amount of $RG\cdot S_1$ due to the dissociation (step 5, Fig. 3). $RG\cdot S_1$ cannot be produced in the amount exceeding the amount of $RG\cdot S_2$ due to the GDP/GTP-exchange process (step 2, Fig. 3), since the action of agonist must lead to the cellular response. Therefore K_1 should be negligible. The expression for $(RG\cdot S_2)$ is:

$$(RG\cdot S_2) = \frac{(R_0) - (B_0)(1 + K_2 z)}{1 + K_1 z} \quad (A.4)$$

Plugging it into equation for (B_0) and introducing variable $y = [(R_0 - (B_0)(1 + K_2 z)]/(R_0)$ (a share of unoccupied receptors) we arrive at the equation:

$$\frac{dy}{dt} - y\left(\frac{k_1(H_0)(1 + K_2 z)}{1 + K_1 z}\right) + y\left(\frac{z'}{z}\right) \quad (A.5)$$

where z' is the time derivative of z. The second term in Eq. (A.5) is much smaller than the first one. In fact, we get $z'/z = [(\Delta S_1)'/(S_1)] + [(\Delta S_2)'/(S_2)]$, where $(\Delta S_1)'$ and $(\Delta S_2)'$ are the time derivatives of (ΔS_1), (ΔS_2). Upon differentiation, these quantities (more precisely the multiplicative constants *a, b, c, d*) are multiplied by the numerical factor $\lambda_1 = \lambda \pm (\sqrt{D}/2) = 1/2\{[-(2k_t + k^{**}_{cat}(E_{20})] \pm \sqrt{k^{**}_{cat}(E_{20})[k^{**}_{cat}(E_{20}) - 4k^*K)]}\}$ that at least two to three orders of magnitude smaller than (*a, b, c, d* ($k_t \sim 10^{-3}$–10^{-5} s^{-1}, $k^{**}_{cat} \sim 10^2$–10^{-2} M^{-1} s^{-1} and $(E_{20}) \sim 10^{-5}$–10^{-6} M). Hence z'/z is $\sim 10^{-2}$–10^{-5}.

Consider now the numerical factor in the first term of Eq. (A.5). Function z reflects the current ratio of the concentrations of GTP and GDP, and is expected to vary by approx. 10^{-1}–10. Therefore $K_1 z \ll 1$, and we can neglect $K_1 z$ in the denominator. In the numerator $k_1 \sim 10^7$–10^9 M^{-1} s^{-1} and $(H_0) = 10^{-9}$ M. G protein has the preference for binding with GTP rather than with GDP. On the other hand, GTP and GDP are substrates with similar structures, and therefore this preference cannot be large in terms of the numerical value of the equilibrium constant K_2 (step 2, Fig. 3). These considerations lead us to think that $K_2 \sim 1$–10 at its minimum. The overall order of the magnitude of the multiplicative factor in the first term of Eq. (A.5) is 10^{-2}–1. Therefore we can neglect the second term in Eq. (A.5). This allows us to integrate Eq. (A.5) and obtain an equation that governs the time dependence of the concentration of the hormone–receptor complex (B_0):

$$[B_0(t)] = \frac{(R_0)(1 - e^{-k_1(H_0)[t + K_2 Z(t)]})}{1 + K_2 z(t)} \tag{A.6}$$

where $Z(t)' = z(t)$. The equation for (B_1) can be integrated, and we obtain $[B_1(t)] = [B_0(t)] z K_2 e^{-k_{-1}t}$. For the total concentration of the hormone–receptor complex $[B(t)] = [B_0(t)] + [B_1(t)]$ we obtain:

$$[B(t)] = (B_{ns}) + \frac{(R_0)(1 - e^{-k_1(H_0)(t + K_2 Z(t)}}{1 + K_2 z(t)} \times (1 + K_2 z(t) e^{-k_{-1}t}) \tag{A.7}$$

We must take into account the process of receptors inactivation with the kinetic constant k_{in}. This process is very slow, and the effect of inactivation can be experimentally observed after 30–40 min have elapsed from the moment of the addition of the agonist to the system of native cells. We absorb it into the basic equation for the time evolution of the total concentration of the hormone–receptor complex. The desired expression is

$$[B(t)] = (B_{ns}) + \frac{(R_0) e^{-k_{in}t}(1 - e^{-k_1(H_0)[t + K_2 Z(t)]})}{1 + K_2 z(t)} \times \left[1 + K_2 z(t) e^{-k_{-1}t}\right] \tag{A.8}$$

Appendix 2: Derivation of the system of equations for (S_1) and (S_2)

Differential equations governing the time evolution of concentrations of GTP and GDP (S_1), (S_2) are

$$\frac{d(S_1)}{dt} = k^{**}_{cat}(E_{20})(S_2) + k^1_t\left[(S^0_1) - (S_1)\right]$$

$$\frac{d(S_2)}{dt} = k^*(AG^* \cdot S_1) - k^{**}_{cat}(E_{20})(S_2) + k^2_t\left[(S^0_2) - (S_2)\right] \tag{B.1}$$

We also need the conservation equations for (S_1), (S_2) and (A):

$$(S_{10}) = (S_1) + (G \cdot S_1) + (G^* \cdot S_1) + (AG^* \cdot S_1) + (RG \cdot S_1) + (HRG \cdot S_1) \tag{B.2}$$

$$(S_{20}) = (S_2) + (G \cdot S_2) + (RG \cdot S_2) + (HRG \cdot S_2) \tag{B.3}$$

$$(A_0) = (A) + (AG^* \cdot S_1) \tag{B.4}$$

Neglecting in Eq. (B.3) the concentrations of $RG \cdot S_2$ and $HRG \cdot S_2$, and in Eq. (B.2), the concentrations of $RG \cdot S_1$ and $HRG \cdot S_1$ (see Section 3.1). The cellular concentration of the effector (A) is close to the concentration of G protein (~

10^{-5}–10^{-6} M). We can also neglect the concentration of the active form of the effector $AG^*\cdot S_1$ in B.2. Then the conservation equations for (S_1) and (S_2) are: $(S_{10}) = (S_1) + (G\cdot S_1) + (G^*\cdot S_1)$ and $(S_{20}) = (S_2) + (G\cdot S_2)$. We express the concentration of the active form of effector via the equilibrium constants K_3 and K_4 as (Fig. 3, steps 7 and 8)

$$(AG^*\cdot S_1) = K_3K_4(A)(G\cdot S_1) \tag{B.5}$$

The expression for $(G^*\cdot S_1)$ is (Fig. 3, step 7):

$$(G\cdot S_1) = \frac{(S_{10}) - (S_1)}{1 + K_3} \tag{B.6}$$

Plugging the Eq. (B.6) into the Eq. (B.5) we obtain $AG^*\cdot S_1 = [K_3K_4(A)(S_{10}) - (S_1)]/(1 + K_3)$. It is not unreasonable to assume that only a small amount of amplifier is involved into the extracellular signal transduction, i.e. the concentration of the active form of effector is small in comparison with that of free effector, i.e. $(A) = (A_0)$. Then we have:

$$(AG^*\cdot S_1) = \frac{K_3K_4(A_0)[(S_{10}) - (S_1)]}{1 + K_3} \tag{B.7}$$

Plugging this expression into the system (B.1) and making algebraic rearrangements, we finally obtain the system (10).

Appendix 3: Linear stability of the system (10)

A solution for (S_1) and (S_2) can be written in the form:

$$(S_1) = (S_1^*) + (\Delta S_1) \text{ and } (S_2) = (S_2^*) + (\Delta S_2) \tag{C.1}$$

where (S_1^*), (S_2^*) are fixed points, that are found to be

$$(S_1^*) = \frac{k_{cat}^{**}(E_{20})[k_t + k^*K(S_{10})] + [k_{cat}^{**}(E_{20}) + k_t]k_t(S_{10})}{k_t[k_{cat}^{**}(E_{20}) - k_t] + k^*k_{cat}^{**}K(E_{20})}$$

$$(S_2^*) = \frac{(k_t)^2(S_{20})}{k_t[k_{cat}^{**}(E_{20}) - k_t] + k^*k_{cat}^{**}K(E_{20})} \tag{C.2}$$

For (S_1^*) and (S_2^*) to be positive definite, the condition $k_{cat}^{**}(E_{20}) > k_t$ must be satisfied. Here (ΔS_1), (ΔS_2) are small variations in the concentrations of GTP and GDP around fixed points, and equations for (ΔS_1), (ΔS_2) are

$$\frac{d(\Delta S_1)}{dt} = -k_t(\Delta S_1) + k_{cat}^{**}(E_{20})(\Delta S_2)$$

$$\frac{d(\Delta S_2)}{dt} = -k^*K(\Delta S_1) - [k_{cat}^{**}(E_{20}) + k_t](\Delta S_2) \tag{C.3}$$

The roots of the characteristic equation are $\lambda_{1,2} = \lambda \pm (\sqrt{D}/2)$, where $\lambda = -[k_t + \frac{1}{2}k_{cat}^{**}(E_{20})]$ and $D = k_{cat}^{**}(E_{20})(k_{cat}^{**}(E_{20}) - 4k^*K)$ is the determinant. The general solution is

$$(S_1) = (S_1^*) + (\Delta S_1) = (S_1^*) + ae^{\lambda_1 t} + be^{\lambda_2 t}$$

$$(S_2) = (S_2^*) + (\Delta S_2) = (S_2^*) + ce^{\lambda_1 t} + de^{\lambda_2 t} \tag{C.4}$$

where constants a, b, c and d are given by

$$a = \frac{[k_{cat}^{**}(E_{20}) - \sqrt{D}]\{\Delta_1[-2k^*K + k_{cat}^{**}(E_{20}) + \sqrt{D}] - \Delta_2[k_{cat}^{**}(E_{20}) + \sqrt{D}]\}}{2\{[k_{cat}^{**}(E_{20}) + \sqrt{D}][k_{cat}^{**}(E_{20}) - \sqrt{D} - 2k^*K] + [k_{cat}^{**}(E_{20}) - \sqrt{D}][k_{cat}^{**}(E_{20}) + \sqrt{D} - 2k^*K]\}} \tag{C.5}$$

$$b = \frac{[k_{cat}^{**}(E_{20}) + \sqrt{D}]\{\Delta_1[2k^*K - k_{cat}^{**}(E_{20}) + \sqrt{D}] - \Delta_2[k_{cat}^{**}(E_{20}) + \sqrt{D}]\}}{2\{[k_{cat}^{**}(E_{20}) + \sqrt{D}][k_{cat}^{**}(E_{20}) - \sqrt{D} - 2k^*K] + [k_{cat}^{**}(E_{20}) - \sqrt{D}][k_{cat}^{**}(E_{20}) + \sqrt{D} - 2k^*K]\}} \tag{C.6}$$

$$c = \frac{[k^{**}_{cat}(E_{20}) - \sqrt{D} - 2k^*K]}{2\{[k^{**}_{cat}(E_{20}) + \sqrt{D}\,][k^{**}_{cat}(E_{20}) - \sqrt{D} - 2k^*K] + [k^{**}_{cat}(E_{20}) - \sqrt{D}\,][k^{**}_{cat}(E_{20}) + \sqrt{D} - 2k^*K]\}} \times \{\Delta_1[2k^*K - k^{**}_{cat}(E_{20}) - \sqrt{D}\,] + \Delta_1[k^{**}_{cat}(E_{20}) + \sqrt{D}\,]\} \quad \text{(C.7)}$$

$$d = \frac{[k^{**}_{cat}(E_{20}) + \sqrt{D} - 2k^*K]}{2\{[k^{**}_{cat}(E_{20}) + \sqrt{D}\,][k^{**}_{cat}(E_{20}) - \sqrt{D} - 2k^*K] + [k^{**}_{cat}(E_{20}) - \sqrt{D}\,][k^{**}_{cat}(E_{20}) + \sqrt{D} - 2k^*K]\}} \times \{\Delta_1[2k^*K - k^{**}_{cat}(E_{20}) + \sqrt{D}\,] - \Delta_1[k^{**}_{cat}(E_{20}) - \sqrt{D}\,]\} \quad \text{(C.8)}$$

and $\Delta_1 = S_{10} - S_1^*$, $\Delta_2 = S_{20} - S_2^*$. In terms of the kinetic and equilibrium parameters we get the condition for the appearance of various kinetic regimes in the time evolution of GTP and GDP:

$$\frac{k^{**}_{cat}}{k^*} \left\{ \begin{matrix} > \\ = \\ < \end{matrix} \right\} \left(\frac{4K_3K_4}{1+K_3} \right) \frac{(A_0)}{(E_{20})} \rightarrow \left\{ \begin{matrix} 2-\text{exponential} \\ 1-\text{exponential} \\ \text{oscillatory} \end{matrix} \right\} \text{kinetics} \quad \text{(C.9)}$$

For the oscillatory kinetics the expressions (C.4) become

$$(S_1) = (S_1^*) + e^{\lambda t} \times \left\{ \sqrt{(\text{Re } a + \text{Re } b)^2 + (\text{Im } a - \text{Im } b)^2} \times \sin\left[\omega t + \arctan\left(\frac{\text{Re } a + \text{Re } b}{\text{Im } a - \text{Im } b} \right) \right] \right\}$$

$$(S_2) = (S_2^*) + e^{\lambda t} \times \left\{ \sqrt{(\text{Re } c + \text{Re } d)^2 + (\text{Im } c - \text{Im } d)^2} \times \sin\left[\omega t + \arctan\left(\frac{\text{Re } c + \text{Re } d}{\text{Im } c - \text{Im } d} \right) \right] \right\} \quad \text{(C.10)}$$

Here the expressions under square roots are the amplitudes, and the arctangent terms are phases of oscillations. The frequency of oscillations is given by

$$\omega = \frac{\sqrt{D}}{2} = \tfrac{1}{2}\{k^{**}_{cat}(E_{20})[k^{**}_{cat}(E_{20}) - 4k^*K]\}^{1/2} \quad \text{(C.11)}$$

When the regime is biexponential, the solution (C.4) is stable, provided that both roots are negative, i.e.

$$\lambda_{1,2} = \tfrac{1}{2}\Big\{ -(2k_t + k^{**}_{cat}(E_{20})) \pm \sqrt{k^{**}_{cat}(E_{20})[k^{**}_{cat}(E_{20}) - 4k^*K]}\Big\} < 0 \quad \text{(C.12)}$$

If the regime is single exponential (oscillatory), the solution (C.4) [solution (C.10)] is stable, if both equal roots are negative, i.e.

$$\lambda_1 = \lambda_2 = -\tfrac{1}{2}[2k_t + k^{**}_{cat}(E_{20})] < 0 \quad \text{(C.13)}$$

It can be shown that the conditions (C.12) and (C.13) are always satisfied.

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