Consequences of Hormone-Induced Desensitization of Adenylate Cyclase in Intact Cells

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A hypothesis on the role of the hormone-induced desensitization of adenylate cyclase is proposed. It is suggested that the desensitization process could provide the cell with a highly efficient cyclic AMP system for transmitting hormone stimulus without requiring a large energy consumption. Theoretical considerations show that in fact the desensitization phenomenon allows the cyclic AMP system to present a good compromise between the efficiency and economy requirements of the cells.

The concentration of cyclic AMP in the intact cell is regulated by the rates of synthesis and disposal. Cyclic AMP synthesis is catalysed by adenylate cyclase, whereas cyclic AMP removal is due to phosphodiesterase activity combined with the escape of cyclic AMP from the cell. It is now generally accepted that in many systems a wide variety of hormone-induced effects are triggered by the increase of intracellular cyclic AMP caused by the alteration of either synthesis or disposal. The theoretical analysis proposed here will deal only with hormone-controlled cyclic AMP synthesis, i.e. we assume that hormone activates adenylate cyclase.

Depending on the system, the cyclic AMP accumulation resulting from a hormone stimulus may exhibit monotonous or biphasic kinetics. Monotonous kinetics can be described as a continuous cyclic AMP accumulation from the basal steady state up to the stimulated steady state, the value of which is fixed by the relative activities of cyclic AMP synthesis and degradation. In fact such a description is usually inadequate to describe cyclic AMP accumulation in cells, but an example of a system that does accumulate cyclic AMP monotonously is pigeon erythrocytes subjected to stimulation by adrenaline (Davoren & Sutherland, 1963). Biphasic kinetics are far more commonly met in cyclic AMP-accumulation time courses. This type of kinetic description is observed in both cultured cells (Barber *et al.*, 1978; Su *et al.*, 1976; Terasaki *et al.*, 1978) and isolated cells or tissues (Kakuchi &

Rall, 1968) under stimulation from a variety of hormones. In this case there is a sharp increase in cyclic AMP, followed by a slow decline to a stimulated steady state. This kind of kinetics has been interpreted for many systems as the consequence of the hormone-induced desensitization of adenylate cyclase. It has been shown that adenylate cyclase is rapidly maximally activated and that the activity subsequently declines to a lower stationary value (Clark & Butcher, 1979). Though the exact molecular mechanism is still unknown, a phenomenological description of adenylate cyclase desensitization can be given (Barber *et al.*, 1978).

The exact role of the desensitization phenomenon has not been defined so far. It may be suggested that desensitization of adenylate cyclase could protect the cell against prolonged hormone stimulation or against stimulation with too high a concentration of hormone. However, such a hypothesis could be valid only if the stimulated steady state of cyclic AMP, the value of which is between the transient maximal value and the basal steady state, is not sufficiently high for triggering all the cellular effects. For some systems it has been observed that only a small increase of intracellular cyclic AMP is all that is necessary for induction of the protein kinase activation and the subsequent effects (Drummond *et al.*, 1977; Schwoch & Hütz, 1977; Sala *et al.*, 1979). It seems thus of prime interest to verify if in the cells exhibiting the desensitization process the ultimate effects are still present when the cyclic AMP

concentration has reached the stimulated steady state. The purpose of this analysis is to demonstrate that a system exhibiting desensitization and operating within defined functional constraints is both more economical and more efficient than classical monophasic systems.

Rationale

The concentration-response relationship between cyclic AMP concentration and the physiological effects may be illustrated as in Fig. 1. We can suppose that there exist two critical cyclic AMP concentrations, one of which is $A_{stim.}$, representing the lower limit of cyclic AMP concentration that can induce the full effect. $A_{stim.}$ is assumed to account for a sufficiently high safety margin such that cyclic AMP variations unrelated to the hormone action are buffered and thus do not greatly alter the final effect of hormone. The other critical value, $A_{inact.}$, is an upper limit under which cyclic AMP concentration may not induce any significant effect.

The main hypothesis of this analysis is that the cyclic AMP system is subject to particular kinetic restraints. In other words, the kinetics of cyclic AMP accumulation should be more or less rapid depending on the physiological role of the cell. The kinetic requirements that a cell must fulfil could be defined as follows.

(1) The cyclic AMP concentration must reach $A_{stim.}$ at a critical time ($t_{stim.}$) after the beginning of the hormone stimulation and must remain above this value as long as stimulation persists.

Fig. 1. Semi-logarithmic representation of the relationship between the cyclic AMP effect and the cyclic AMP concentration

$A_{stim.}$ is the value that the cyclic AMP concentration should attain to ensure full effect with a sufficiently high safety margin. Cyclic AMP concentration below the $A_{inact.}$ value would not induce any significant effects.

(2) The cyclic AMP concentration must fall below the $A_{inact.}$ value at a critical time ($t_{inact.}$) after the stimulation is removed.

(3) In order to save energy, i.e. the consumption of ATP and GTP needed for cyclic AMP synthesis, the activity of adenylate cyclase must be as low as possible.

Since the two first kinetic requirements imply a rather high adenylate cyclase activity, the cyclic AMP-metabolism system should exhibit characteristics that could account for the economy requirement. The kinetic requirements are illustrated in Fig. 2. It is assumed that in the absence of hormone the basal cyclic AMP concentration is zero. The kinetic requirements imply that the time course of cyclic AMP concentration may not pass through the prohibited shaded areas. The purpose of the proposed analysis is to compare the respective characteristics of the systems exhibiting either monotonous or biphasic cyclic AMP accumulation and accounting for the requirements defined below.

Definition of the models

The cyclic AMP-synthesis and -disposal rates are respectively referred to as v_s and v_d . If A is the intracellular concentration of cyclic AMP, the variation of cyclic AMP concentration with respect

Fig. 2. Representation of the kinetic requirements for the cyclic AMP accumulation and decrease

The requirements 1 and 2 (see the text) are fulfilled if the time-course of cyclic AMP concentration does not pass through the shaded areas 1 and 2. It is assumed that in the absence of hormone stimulation the basal cyclic AMP concentration is zero. Typical cyclic AMP time courses accounting for the kinetic requirements are shown: monotonous (----) and biphasic (....) cyclic AMP accumulation during hormone stimulation (hormone H is present as indicated along the abscissa) and cyclic AMP decrease after stimulation cut-off.

to time is given by

$$\frac{dA}{dt} = v_s - v_d \quad (1)$$

In the case of monotonous kinetics (model 1), v_s is a constant and is equal to k_s as long as the hormone is biologically active. As has been demonstrated in some systems (Su *et al.*, 1976; Barber *et al.*, 1978), the disposal rate can be described as a function of cyclic AMP concentration. Eqn. (1) then becomes

$$\frac{dA}{dt} = k_s - k_{d1}A \quad (2)$$

where k_{d1} is the first-order parameter describing the cyclic AMP degradation process. Since $A(t=0) = 0$, the solution is

$$A(t) = \frac{k_s}{k_{d1}} (1 - e^{-k_{d1}t}) \quad (3)$$

In the case of biphasic kinetics (model 2), the synthesis rate v_s is a function of time, and it is presumed that v_s exponentially varies from the initial high value k_1 to the final low value k_f , i.e.

$$v_s = k_f + (k_1 - k_f)e^{-\gamma t} \quad (4)$$

where γ is a constant describing the rate of adenylate cyclase desensitization. Eqn. (1) thus becomes

$$\frac{dA}{dt} = k_f + (k_1 - k_f)e^{-\gamma t} - k_{d2}A \quad (5)$$

where k_{d2} is defined as k_{d1} in model 1, and since $A(t=0) = 0$, the solution is

$$A(t) = \frac{k_1 - k_f}{k_{d2} - \gamma} e^{-\gamma t} - \left[\frac{k_f}{k_{d2}} + \frac{k_1 - k_f}{k_{d2} - \gamma} \right] e^{-k_{d2}t} + \frac{k_f}{k_{d2}} \quad (6)$$

The energy consumed by the cyclic AMP-metabolic system during the time of stimulation is directly related to the cyclic AMP synthesis. We can thus define the mean rate of energy consumption, C , as the integral with respect to time of the adenylate cyclase activity divided by the time:

$$C = \frac{1}{t} \int_0^t v_s dt \quad (7)$$

For model 1, describing monotonous cyclic AMP accumulation, the mean rate of energy consumption C is just the adenylate cyclase activity:

$$C_1 = k_s \quad (8)$$

For model 2, which is characterized by the adenylate cyclase desensitization, we obtain:

$$C_2 = \frac{1}{t} \int_0^t [k_f + (k_1 - k_f)e^{-\gamma t}] dt \quad (9)$$

and thus

$$C_2 = k_f + \frac{k_1 - k_f}{\gamma} \left(\frac{1 - e^{-\gamma t}}{t} \right) \quad (10)$$

Theoretical Analysis of the Models

The introduction of the kinetic requirements into the two models can be done by fitting the two critical points ($A_{stim.}$, $t_{stim.}$) and ($A_{inact.}$, $t_{inact.}$) with the time course of cyclic AMP concentration. For model 1, these two constraints unequivocally fix the values of the two parameters k_s and k_{d1} . For model 2 there is an infinity of numerical values for the four parameters k_1 , k_f , γ and k_{d2} that can fit the two critical points. The only supplementary constraints are that the stimulated steady state A_2^+ given by k_f/k_{d2} cannot be lower than the critical concentration $A_{stim.}$:

$$A_2^+ = \frac{k_f}{k_{d2}} > A_{stim.} \quad (11)$$

The values of the parameters are computed in the Appendix.

In the case of model 2, the mean rate of energy consumption becomes mainly dependent on the k_f value as the time increases. In order to minimize this consumption, the system must exhibit the lowest possible value for k_f and thus the system takes advantage of a low cyclic AMP steady state. Thus we can expect that the more economical system described by model 2 is characterized by a steady-state value A_2^+ equal to the critical value $A_{stim.}$. On the other hand, model 1 always exhibits a steady state A_1^+ higher than this critical value because of the monotonous shape of the cyclic AMP time course. It is demonstrated in the Appendix that, because of the respective values of the steady state in models 1 and 2, the adenylate cyclase activity k_s for model 1 is always higher than the final value k_f of adenylate cyclase activity for model 2. Moreover, it is also demonstrated in the Appendix that the first-order constant for the disposal process is higher in model 1 (k_{d1}) than in model 2 (k_{d2}). Therefore it is always possible to find an initial value k_1 of the adenylate cyclase activity (model 2) that is not greater than the adenylate cyclase activity k_s (model 1). Thus there always exists a set of parameters for model 2 such that the biphasic kinetics of cyclic AMP accumulation are more economical than monotonous kinetics (model 1).

Numerical Simulation of the Models

In order to simulate the cyclic AMP time course for both models, we have to choose numerical values

for the critical points characterizing the cell requirements. The values are expressed in arbitrary units:

$$\begin{aligned} A_{\text{stim.}} &= 1 \\ t_{\text{stim.}} &= 1 \\ A_{\text{inact.}} &= 0.1 \\ t_{\text{inact.}} &= 10 \end{aligned}$$

It should be emphasized that these numerical values could be changed without affecting the conclusions deduced from the simulation.

By using the equations obtained in the Appendix, we can compute the parameters of model 1. We obtain

$$\begin{aligned} k_s &= 1.16 \\ k_{d_1} &= 0.35 \end{aligned}$$

and the steady state is then

$$A_1^+ = 3.31$$

For model 2 we find

$$\begin{aligned} k_f &= 0.23 \\ k_{d_2} &= 0.23 \end{aligned}$$

The stimulated steady state A_2^+ is equal to a $A_{\text{stim.}}$, since such a value provides the lowest value for k_f , as shown above. The values of the other parameters are shown in Table 1. All the proposed solutions take the cell kinetic requirements into account.

If the initial adenylate cyclase activity k_i of model 2 is equal to the adenylate cyclase activity k_s of model 1, we can see that, as expected, the mean energy-consumption rate C_2 for model 2 is at all times lower than the mean energy consumption rate

C_1 for model 1. If we now increase the value of the parameter γ , i.e. the rate of adenylate cyclase desensitization, then the k_i value must be increased as well, because the cyclic AMP time course must pass through the critical point ($A_{\text{stim.}}$, $t_{\text{stim.}}$). Because of the k_i increase, we can expect that the mean rate of energy consumption will be higher for small times, but, since the rate of adenylate cyclase desensitization is also increased, the adenylate cyclase activity reaches much more rapidly the low final value k_f . Therefore the mean rate of energy consumption should be lower at longer times. This speculation is confirmed by the theoretical simulation, as shown in Table 1.

The results shown in Table 1 also demonstrate that for model 2 the energy consumption, i.e. the amount of cyclic AMP synthesized, is one-half of the consumption in model 1 for a short time, i.e. about two-fold the time required for attaining the transient maximal cyclic AMP concentration.

Analysis of an Experimental Cyclic AMP Time Course

The analysis of the advantage to a cell of the hormone-induced adenylate cyclase desensitization is mainly based on the postulate that a cell should work in a way that is both efficient and economical. We arrived at the conclusion that a biphasic cyclic AMP-accumulation process can fulfil certain operational requirements while consuming less energy than a monotonous cyclic AMP accumulation does, when both systems satisfy the cell requirements for activation and inactivation rates. In order to apply the theoretical considerations that we have presented here, we have taken as an example the cyclic AMP time course obtained with human diploid fibroblasts (WI-38) stimulated by prostaglandin E_1 . These cells present homologous desensitization, and the kinetics of cyclic AMP accumulation have been described (Barber *et al.*, 1978). A typical experimental result is shown in Fig. 3. The theoretical fitting of these data by model 2 led to the following values for the kinetic parameters: $k_i = 0.015$ fraction conversion of ATP into cyclic AMP/min; $k_f = 0.0077$ fraction conversion of ATP into cyclic AMP/min; $\gamma = 0.15$ /min; $k_{d_2} = 0.35$ /min. The cyclic AMP-stimulated steady state is equal to $k_f/k_{d_2} = 0.022$ fraction conversion of ATP into cyclic AMP. The theoretical cyclic AMP time course described with these parameter values is represented by the solid line in Fig. 3. If we assume as before that the critical stimulated cyclic AMP concentration is just equal to the steady state and that $A_{\text{inact.}} = 0.1$, $A_{\text{stim.}}$, then we find that $t_{\text{stim.}} = 2.381$ min and $t_{\text{inact.}} = 6.579$ min.

In such circumstances, the system characterized by monotonous kinetics, i.e. model 1, can account

Table 1. Simulation of model 1 (monotonous kinetics) and model 2 (biphasic kinetics)

The mean energy-consumption rate C has been computed for different values of the parameters characterizing the models. In each case, the cyclic AMP time course passes through the following critical points: $A_{\text{stim.}} = 1$, $t_{\text{stim.}} = 1$ for stimulation; $A_{\text{inact.}} = 0.1$, $t_{\text{inact.}} = 10$ for inactivation.

Model 1

k_s	k_{d_1}	C_1		
		$t = 1$	$t = 10$	$t = 50$
1.16	0.35	1.16	1.16	1.16

Model 2

k_i	k_f	γ	k_{d_2}	C_2		
				$t = 1$	$t = 10$	$t = 50$
1.16	0.23	0.1	0.23	1.12	0.82	0.42
1.27	0.23	0.3	0.23	1.12	0.56	0.30
1.66	0.23	1	0.23	1.14	0.37	0.26
5	0.23	5	0.23	1.19	0.33	0.25
20	0.23	20	0.23	1.22	0.33	0.25

Fig. 3. Cyclic AMP time course in WI-38 cells stimulated by prostaglandin E₁

The adenine nucleotide pools of cells were pre-labelled with [³H]adenine to facilitate analysis (Su *et al.*, 1976). Prostaglandin E₁ was added at zero time to a final concentration of 5.7 μM. At the indicated times the incubation medium was withdrawn and the incubation was stopped by addition of cold 5% (w/v) trichloroacetic acid. Accumulation of cyclic AMP is expressed as a fraction of the cellular ATP (Su *et al.*, 1976). The solid and dashed lines are, respectively, the Cartesian representations of model 2 and model 1 by using the parameters quoted in the text.

Table 2. Comparison of the mean rate of energy consumption for WI-38 cells and for theoretical monotonous kinetics

The parameters required by the monotonous model to fulfil the required kinetic restraints are: $A_{stim.} = 0.022$ fraction conversion, $t_{stim.} = 2.381$ min; $A_{inact.} = 0.022$ fraction conversion, $t_{inact.} = 6.579$ min.

<i>t</i> (min)	WI-38 cells	Monotonous kinetics
1	0.0145	0.0146
5	0.0128	0.0146
10	0.0115	0.0146
20	0.0100	0.0146
50	0.0087	0.0146

for these kinetic requirements if $k_s = 0.0146$ fraction conversion of ATP into cyclic AMP/min and $k_d = 0.412$ /min. The steady state is thus equal to 0.0354 fraction conversion. The theoretical cyclic AMP time course corresponding to this model is represented by the dashed line in Fig. 3.

Table 2 shows the values of the rate of energy consumption for WI-38 cells and the corresponding monotonous kinetic model. As expected, the values characterizing cyclic AMP accumulation in WI-38 cells are significantly lower than the rates needed for

a mode in which the desensitization of adenylate cyclase does not occur.

Discussion and Conclusion

The widespread occurrence of hormone-induced desensitization of adenylate cyclase has prompted us to search for a possible physiological role of this phenomenon. Since the concentration of cyclic AMP at the stimulated steady state, which is drastically lower than the transient maximal value, would be sufficient to induce the cellular effects in several systems, the role of the desensitization process as a protection of the cell against excessive stimulation remains questionable. Among the alternative hypothesis, the one developed here was based on the fact that the desensitization process could provide the cell with a cyclic AMP system that is both efficient and economical. The concentration of cyclic AMP must increase or decrease at such a rate that the temporal requirements for hormonal action are satisfied. To achieve this end, the kinetics of cyclic AMP metabolism must frequently be rapid. If monotonous kinetics were employed by the cell, energy consumption would be high. Conversely, the theoretical analysis has shown that the kinetic requirements of the cell may be accounted for in a more economical way if the system exhibits a hormone-induced adenylate cyclase desensitization.

As shown above, the mean rate of energy consumption is a decreasing function of time in the case of model 2. Since it is assumed that the duration of the stimulation is high enough for desensitization to occur, we have to compare the values of the mean energy-consumption rate obtained at a time where the system is desensitized ($t > 1/\gamma$), i.e. the adenylate cyclase activity is close to the final activity k_f . In these conditions, the results presented in Table 1 show that because of a high initial adenylate cyclase activity k_i and a high desensitization rate γ , the system is as economical as possible. However, numerical results suggest that the mean rate of energy consumption decreases more and more slowly as k_i and γ increase. In other words, the system does not need k_i and γ higher than a certain limit, which is in the considered case about 5 for both parameters.

In conclusion, it should be advantageous for the cell if the cyclic AMP system were characterized by high k_i and γ and low k_f and k_d , as such relative values allow relatively low energy consumption. However, such requirements may be difficult for the cell to fulfil in molecular terms, with the real system being a compromise between rapid response and economical use of energy.

The quantitative description of an experimental system such as prostaglandin E₁-stimulated fibroblasts exemplified our theoretical considerations. It

seems obvious that such an analysis will be extended to desensitization phenomena in other energy-consuming hormonal and neuro-transmitter systems, and even to some biphasic physiological processes.

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APPENDIX

For model 1, the two parameters k_s and k_{d_1} must account for the two following equations, which express the fact that the cyclic AMP time course passes through the two critical points:

$$A_{\text{stim.}} = \frac{k_s}{k_{d_1}} (1 - e^{-k_{d_1} t_{\text{stim.}}}) \quad (\text{A1})$$

$$A_{\text{inact.}} = \frac{k_s}{k_{d_1}} e^{-k_{d_1} t_{\text{inact.}}} \quad (\text{A2})$$

thus k_{d_1} is given by

$$1 - e^{-k_{d_1} t_{\text{stim.}}} - \frac{A_{\text{stim.}}}{A_{\text{inact.}}} e^{-k_{d_1} t_{\text{inact.}}} = 0 \quad (\text{A3})$$

and k_s is then

$$k_s = \frac{A_{\text{inact.}} k_{d_1}}{e^{-k_{d_1} t_{\text{inact.}}}} \quad (\text{A4})$$

For model 2, the stimulated steady state A_2^+ can be chosen just equal to the critical value $A_{\text{stim.}}$. If we consider that the time of stimulation is such that the cyclic AMP concentration reaches a value very close to the steady-state value, then k_f and k_{d_2} must fulfil the two following equations:

$$\frac{k_f}{k_{d_2}} = A_{\text{stim.}} \quad (\text{A5})$$

$$A_{\text{inact.}} = \frac{k_f}{k_{d_2}} e^{-k_{d_2} t_{\text{inact.}}} \quad (\text{A6})$$

and thus

$$k_{d_2} = \frac{1}{t_{\text{inact.}}} \ln \left(\frac{A_{\text{stim.}}}{A_{\text{inact.}}} \right) \quad (\text{A7})$$

$$k_f = A_{\text{stim.}} k_{d_2} \quad (\text{A8})$$

It has been demonstrated that the final value (k_f) of adenylate cyclase activity in model 2 is always lower than the constant stimulated adenylate cyclase activity (k_s) in model 1. Since model 1 exhibits monotonous kinetics for cyclic AMP accumulation, it is clear that the cyclic AMP steady state A_1^+ is higher than the critical value $A_{\text{stim.}}$. In model 2, the steady state A_2^+ is equal to the value $A_{\text{stim.}}$ and thus

$$A_1^+ = \frac{k_s}{k_{d_1}} > \frac{k_f}{k_{d_2}} = A_2^+ \quad (\text{A9})$$

or

$$\frac{k_s}{k_f} > \frac{k_{d_1}}{k_{d_2}} \quad (\text{A10})$$

Now the stimulated steady state of cyclic AMP concentration represents the amount of cyclic AMP that has to be hydrolysed for the basal value to be re-attained. Since the critical $A_{\text{inact.}}$ must be attained at the same time $t_{\text{inact.}}$ for both models, it is clear that the disposal rate in model 1 must be higher than the disposal rate in model 2 and thus

$$k_{d_1} > k_{d_2} \quad (\text{A11})$$

Using this result in eqn. (A10), we obtain

$$k_s > k_f \quad (\text{A12})$$