Theoretical analysis of the consequences of cyclic nucleotide phosphodiesterase negative co-operativity

Amplification and positive co-operativity of cyclic AMP accumulation

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Most tissues contain multiple forms of cyclic nucleotide phosphodiesterases (3':5'-cyclic-nucleotide 5'-nucleotidohydrolase, EC 3.1.4.17). Consequently, in most, if not in all, tissues, substrate-velocity curves deviate from Michaelian kinetics and exhibit an apparent negative co-operativity. We have studied the possible theoretical consequences of this property on the quantitative features of cyclic AMP accumulation in response to activation of adenylate cyclase. Negative co-operativity of phosphodiesterases tends to generate a 'positively co-operative' cyclic AMP accumulation curve. It amplifies the stimulation of cyclic AMP accumulation as compared with the stimulation of cyclic AMP synthesis. It enhances the sensitivity of cyclic AMP accumulation to slight variations of phosphodiesterase maximal velocity. It tends to shift the cyclic AMP accumulation curve to higher concentrations of stimulator as compared with the adenylate cyclase activation curve. This accounts for much of the data in the literature of hormonal effects on phosphodiesterase activity. It shows that the characteristics of cyclic nucleotide phosphodiesterases are as important as those of adenylate cyclase in determining the response of the system.

In several tissues, such as the adrenal (Rodbard, 1974) or the thyroid (Boeynaems *et al.*, 1974), the concentration-response curve relating cyclic AMP accumulation to the concentration of adenylate cyclase-stimulating hormone is positively co-operative (in the purely phenomenological sense), i.e. this curve extends over less than two orders of magnitude and is characterized by a Hill coefficient (Hill, 1913) greater than 1. The amount of intracellular accumulation of this messenger and the quantitative characteristics of this concentration-response curve depend on the kinetic properties of the hormone-responsive adenylate cyclase on one hand, and on those of the phosphodiesterases on the other hand. The relationship between the concentration of hormone and adenylate cyclase activation is either negatively co-operative (Pochet *et al.*, 1973), non-co-operative (Steer & Levitzki, 1975) or positively co-operative (Rodbard *et al.*, 1974), depending on the hormone and the tissue. On the contrary, in most if not in all tissues, the rate of cyclic AMP hydrolysis measured in crude extracts depends in a negatively co-operative fashion on the concentration of cyclic AMP. This could be due to different properties of the enzyme(s): existence of

multiple molecular forms (Russell *et al.*, 1973), sequential negative co-operativity (Russell *et al.*, 1972), or coupling between substrate binding and equilibrium between monomeric and polymeric forms (Pichard & Cheung, 1976). The kinetics of a system of two enzymes with different characteristics and those of a single, truly negatively co-operative, enzyme can be described by the same equations (Boeynaems *et al.*, 1974). Furthermore, in most systems, both 'low- K_m ' and 'high- K_m ' components of phosphodiesterase activity are expected to contribute to cyclic AMP hydrolysis at the micromolar concentration of this nucleotide that exists in the cell (Wells & Hardman, 1977). In the present work, the consequences of the characteristics of phosphodiesterase activity on the cyclic AMP response to stimuli in intact cells are analysed.

Theory

Generation of positive co-operativity in the dose-response curve of cyclic AMP accumulation

Cyclic AMP is synthesized by a ligand (L)-sensitive adenylate cyclase (step I) and hydrolysed by phosphodiesterases (step II). For the sake of

simplicity, let us assume that both processes of adenylate cyclase activation and cyclic AMP hydrolysis can be described by a Hill-type equation. This does not imply any particular enzymic mechanism. In intact cells, cyclic AMP metabolism can thus be described by the following model:

step I:

where V_0 is the basal activity in the absence of ligand (L); V_s is the maximal activity under stimulation; and R and E are the receptor and effector unit of adenylate cyclase;

step II:

Adenylate cyclase activation is described by the equation:

$$v_s = V_0 + V_s \cdot \frac{[L]^{h_s}}{K_s + [L]^{h_s}} \quad (1)$$

where v_s is the rate of cyclic AMP formation, and V_s , K_s and h_s are respectively the maximal velocity, apparent dissociation constant and Hill coefficient for the Hill model applied to cyclic AMP synthesis.

Cyclic AMP hydrolysis by phosphodiesterases is described by the equation:

$$v_D = V_D \cdot \frac{[S]^{h_D}}{K_D + [S]^{h_D}} \quad (2)$$

where V_D , K_D and h_D are respectively the maximal velocity, apparent dissociation constant and Hill coefficient of the Hill model applied to cyclic AMP degradation. In fact, this equation may describe total disposal of cyclic AMP by one or by several enzymes, by an efflux or by any combination of these.

The concentration of cyclic AMP, [S], reaches a steady state when

$$v_s = v_D \quad (3)$$

or

$$V_0 + V_s \cdot \frac{[L]^{h_s}}{K_s + [L]^{h_s}} = V_D \cdot \frac{[S]^{h_D}}{K_D + [S]^{h_D}} \quad (4)$$

In the absence of basal adenylate cyclase activity ($V_0 = 0$), the cyclic AMP steady-state concen-

tration at any concentration of ligand or hormone [L] is expressed by:

$$[S] = \left(\frac{V_s K_D}{V_D - V_s} \right)^{1/h_D} \cdot \left(\frac{[L]^{h_s}}{\frac{V_D K_s}{V_D - V_s} + [L]^{h_s}} \right)^{1/h_D} \quad (5)$$

and its maximal concentration:

$$[S_M] = \left(\frac{V_s K_D}{V_D - V_s} \right)^{1/h_D} \quad (6)$$

The curve relating the concentration of cyclic AMP, [S], to the ligand concentration, [L], (eqn. 5) can be formally characterized by the Hill coefficient, h_A , expressed by the following relationship:

$$h_A = \frac{[L]}{[s] \cdot (1 - [s])} \cdot \frac{d[s]}{d[L]} \quad (7)$$

in which [s] represents the fractional cyclic AMP response and is equal to $[S]/[S_M]$. After derivation of eqn. (5) as a function of [L] and algebraic rearrangement of eqn. (7) with the value of the derivate it follows that the Hill index of cyclic AMP accumulation is given by

$$h_A = \frac{h_s(1 - [s]^{h_D})}{h_D(1 - [s])} \quad [8; \text{see Fig. 1(a)}]$$

It can be easily shown that, when h_D is lower than 1, h_A is higher than h_s and, conversely, h_A is lower than h_s when h_D is higher than 1. Thus, there exists an inverse relation between the co-operativity of the phosphodiesterases and the co-operativity of cyclic AMP accumulation. The Hill coefficient changes along the cyclic AMP accumulation curve from a maximal (or minimal) value to h_s . It is only when cyclic AMP is degraded by one Michaelis enzyme ($h_D = 1$) that h_A becomes equal to h_s .

Rather than defining the Hill index at each point of the concentration-response curve (i.e. at each concentration of cyclic AMP), one may consider the mean Hill index, $\bar{h}_A$, characterizing cyclic AMP accumulation in the interval $[S] = 0.1[S_M]$ to $[S] = 0.9[S_M]$. It is expressed by:

$$\bar{h}_A = \frac{\log 81}{\log \frac{[L]_{0.9\max.}}{[L]_{0.1\max.}}} \quad (9)$$

The ratio $[L]_{0.9\max.}/[L]_{0.1\max.}$ is obtained from eqn. (5), when $[S]/[S_M]$ ratios reached 90 and 10% respectively. Then:

$$\bar{h}_A = h_s \cdot \frac{\log 81}{(\log 9) \cdot h_D + \log[1 - 0.1^{h_D}] - \log[1 - 0.9^{h_D}]}$$

[10; see Fig. 1(b)]

Fig. 1. Influence of the Hill coefficient of phosphodiesterase activity on the Hill coefficient of the dose-response curve for cyclic AMP

(a) The relationship between the ratio of the Hill coefficients describing cyclic AMP accumulation and formation (h_A/h_S) and the fractional cyclic AMP accumulation, $[S]/[S_M]$, is represented according to eqn. (8) for different values of h_D (the Hill coefficient for cyclic AMP catabolism). (b) The mean Hill coefficient characterizing the dose-response curve relating hormone concentration and second messenger accumulation plotted against the Hill coefficient of cyclic AMP catabolism, according to eqn. (10).

As shown in Fig. 1(b) again, there is an inverse relationship between the mean Hill coefficient of cyclic AMP accumulation and the Hill coefficient characterizing the kinetics of cyclic AMP degradation. Similar results are obtained when V_0 is different from 0. In conclusion, negative co-operativity of phosphodiesterase increases the co-operativity of the relation between hormone (ligand) concentration and cyclic AMP accumulation (h_A) and vice versa.

Amplification of cyclic AMP response

Let us assume that the rate of formation of cyclic AMP, or adenylate cyclase activity, (step I) has a basal activity (V_0) in the absence of activating ligand. The factor of stimulation of cyclic AMP synthesis is given by the ratio V_s/V_0 . The steady-state concentration of cyclic AMP is expressed by eqn. (4) and it follows that in the absence of stimulation:

$$[S_0] = \left[\frac{V_0 K_D}{V_D - V_0} \right]^{1/h_D} \quad (11)$$

Under maximal stimulation:

$$[S_M] = \left[\frac{(V_0 + V_s) K_D}{V_D - (V_0 + V_s)} \right]^{1/h_D} \quad (12)$$

and

$$\frac{[S_M]}{[S_0]} = \left[\frac{V_0 + V_s}{V_0} \right]^{1/h_D} \cdot \left[\frac{V_D - V_0}{V_D - V_0 - V_s} \right]^{1/h_D} \quad (13)$$

Whenever $h_D \leq 1$,

$$\frac{[S_M]}{[S_0]} > \left[\frac{V_0 + V_s}{V_0} \right]$$

The fact that steady-state cyclic AMP accumulation (relative to its basal level) is larger than the stimulation of cyclic AMP synthesis (step I) represents an amplification of the hormone signal. This amplification will be maximal when the Hill index, h_D , is low, phosphodiesterase maximal activity compared with V_s and $V_0(V_D - V_0 - V_s)$ is low and the factor of stimulation (step I), V_s/V_0 is large (Fig. 2). Any regulation of phosphodiesterase activity that increases the negatively co-operative kinetic behavior of cyclic AMP disposal will potentiate cyclic AMP accumulation and *vice versa*. In the model considered above, this amplification can be intuitively related to the fact that cyclic AMP degradation enzymes are saturable.

Hormone mid-saturation concentration

The level of hormone that elicits a half-maximal

Fig. 2. Influence of the Hill coefficient of phosphodiesterase activity on the maximal steady-state concentration of cyclic AMP

The ratio of maximal $[S_M]$ and basal $[S_0]$ steady-state concentrations of cyclic AMP are plotted against the Hill coefficient of cyclic AMP catabolism, h_D , according to eqn. (13) ($V_0 = 1$; $V_s = 4$; $V_D = 10$).

Fig. 3. Relationship between the hormone concentration at half-saturation of cyclic AMP accumulation and the Hill coefficient of phosphodiesterase activity

The ratio of hormone concentration at half-saturation of cyclic AMP concentration ($[L]_{A,0.5}$) and at half-maximal activation of adenylate cyclase ($[L]_{S,0.5}$) is represented as a function of the Hill coefficient of cyclic AMP catabolism (h_D) according to eqn. (15) ($h_s = 1$; $V_D = 2V_s$).

stimulatory effect on adenylate cyclase (step I), $[L]_{S,0.5}$, is calculated from eqn. (1) (when $V_0 = 0$):

$$[L]_{S,0.5} = K_s^{1/h_s} \quad (14)$$

The relationship of the steady-state cyclic AMP concentration to hormone concentration, eqn. (5), can be characterized by a hormonal concentration $[L]_{A,0.5}$ at which a half-maximal cyclic AMP concentration is reached. Solving eqn. (5) for $[S] = 0.5 [S_M]$:

$$[L]_{A,0.5} = K_s^{1/h_s} \cdot \left[\frac{V_D}{V_D - V_s} \cdot \frac{(0.5)^{h_D}}{1 - (0.5)^{h_D}} \right]^{1/h_s} \quad (15)$$

Whenever degradation kinetics exhibit negative co-operativity, the cyclic AMP accumulation curve will be shifted to higher concentrations as compared with the synthesis-stimulation curve. This decrease in sensitivity towards the hormone is most striking when V_D and h_D are low. In other words, when cyclic AMP degradation presents no or negative

co-operativity, half-maximal cyclic AMP concentration is reached at a higher concentration of hormone than that eliciting half-maximal cyclase activation. (Fig. 3).

Sensitivity of maximal cyclic AMP accumulation to variations of the kinetic parameters of the phosphodiesterases (K_D or V_D)

The ratio between the relative variations of maximal cyclic AMP steady-state concentrations and the maximal velocity of phosphodiesterase (step II) is obtained from derivation of eqn. (6) and is expressed by:

$$\frac{d[S_M]/[S_M]}{dV_D/V_D} = -\frac{1}{h_D} \cdot \frac{V_D}{(V_D - V_s)} \quad (16)$$

It appears clearly that the negative co-operativity of phosphodiesterase will enhance the relative variation of cyclic AMP accumulation in response to changes of phosphodiesterase maximal velocity, so that small changes of this last parameter could exert a major effect on cyclic AMP concentration (Fig. 4). This sensitivity is also greater when $V_D - V_S$ is low.

On the other hand, the relative variations of cyclic AMP accumulation and the apparent dissociation constant of phosphodiesterase [K_D^{1/h_D} , i.e. the half-saturating substrate concentration of the Hill model used to describe phosphodiesterase activity, eqn. (2)] is expressed by:

$$\frac{\left(\frac{d[S_M]}{[S_M]} \right)}{\left[\frac{d(K_D^{1/h_D})}{K_D^{1/h_D}} \right]} = 1 \quad (17)$$

Negative co-operativity does not enhance the effect on cyclic AMP accumulation of small variations of the apparent affinity constant of phosphodiesterase.

Discussion

Most mammalian systems contain two or more forms of phosphodiesterase ('high and low K_m ' enzymes) (Wells & Hardman, 1977). At micro-molar substrate concentration, the sum of the two enzyme activities governs cyclic AMP degradation, which therefore exhibits negative co-operativity (in the purely phenomenological meaning of the word, i.e. $h_D < 1$). A simple theoretical analysis shows that this property may exert significant quantitative effects on the accumulation of cyclic AMP in cells stimulated by adenylate cyclase-activating ligands.

1. It increases the co-operativity of the relation between the cyclic AMP concentration and the concentration of activating ligand, thus increasing the sensitivity to variations of ligand concentration.

2. It amplifies the stimulation of cyclic AMP accumulation as compared with the stimulation of cyclic AMP synthesis; it amplifies the signal but ensures economy of ATP.

3. It enhances the sensitivity of cyclic AMP accumulation to slight variations of phosphodiesterase maximal velocity, which makes this enzyme an efficient device for regulating cyclic AMP concentration in the cell.

4. On the other hand, it decreases the sensitivity of cyclic AMP accumulation to the actual concentration of hormone (the cyclic AMP accumulation curve is shifted to higher hormone concentrations, as compared with the adenylate cyclase activation curve).

The potential applicability and importance of these theoretical considerations is supported by the quantitative comparison of the cyclic AMP system in dog and horse thyroid. The curve relating cyclic AMP accumulation to thyrotropin concentration is positively co-operative in dog (and horse) thyroid; however no degradation of thyrotropin has been observed under the same conditions (Boeynaems *et al.*, 1973). Horse thyroid contains a lower phosphodiesterase activity than does that of the dog, and its activity is characterized by a lower Hill coefficient. Cyclic AMP accumulation is characterized in the horse thyroid by a larger stimulability (Boeynaems *et al.*, 1974; J. Van Sande, unpublished work), larger Hill coefficient and larger thyrotropin mid-saturation concentration, which corresponds exactly to the predictions of our theoretical analysis (Table 1).

These results help to understand much of the data in the literature. For example, concentration-response curves relating cyclic AMP accumulation to adrenocorticotropin concentration in adrenal cortex (Rodbard, 1974) and to thyrotropin concentration in thyroid (Boeynaems *et al.*, 1974) are characterized by positive co-operativity (Hill coefficient larger than 1). Various mechanisms have

Fig. 4. Influence of the maximal velocity of phosphodiesterase on maximal steady-state accumulation of cyclic AMP

The relationship between maximal steady-state concentration of cyclic AMP ($[S_M]$) and maximal velocity of phosphodiesterase (V_D/V_S) is represented according to eqn. (6) ($V_S = 1$; $K_D = 5$).

Table 1. *Cyclic AMP metabolism in thyroid*
Data were obtained from Boeynaems *et al.* (1974, 1978) and from Pochet *et al.* (1973).

	Horse	Dog
1. Cyclic AMP		
Basal cyclic AMP concentration (pmol/100mg)	26	46
Stimulatory ratio	437	15
$10^9 \times \text{Thyrotropin}_{0.5}$ (M)	6.5	1.1
$\frac{1}{h_A}$	1.8	1.4
2. Cyclic AMP phosphodiesterase		
V_D (pmol/min per mg)	644	2393
$10^4 \times \text{Mean } K_D$ (M)	0.8	0.3
h_D	0.73	0.92
3. Adenylate cyclase		
Basal activity (pmol/15 min per mg)	125	25
Stimulatory ratio	2.7	2.6

been proposed to account for this quantitative feature: allosteric properties of the hormone receptor and/or hormone-sensitive adenylate cyclase activation (Rodbard, 1974); hormone degradation (Cooper & Schulster, 1977); and the existence of an unstirred layer coupled to the degradation of the ligand (Desimone & Price, 1978). We have shown that negative co-operativity of phosphodiesterases could constitute an alternative explanation.

Changes in phosphodiesterase activity after hormone treatment have been reported (Correze *et al.*, 1974; Van Inwegen *et al.*, 1975). The best example is shown with insulin: in liver and adipose tissue, insulin stimulates a particulate negatively-co-operative phosphodiesterase and inhibits the accumulation of cyclic AMP promoted by glucagon or adrenaline (Loten & Sneyd, 1970; Loten *et al.*, 1978). The effects of insulin on phosphodiesterase maximal velocity are small with regard to the effects on cyclic AMP. This apparent discrepancy can be explained by the negative co-operativity of phosphodiesterase activity; we have shown that small effects on V_D induce large changes in cyclic AMP concentrations. More generally, positive co-operativity (Terasaki & Appleman, 1975) or no co-operativity (Russell *et al.*, 1973) of the cyclic AMP phosphodiesterase system has been described; it is therefore easy to understand that phosphodiesterase inhibitors, even those very selective against one enzymic form, may increase cyclic AMP concentrations to very different extents when tested in different systems (Van Sande & Dumont, 1973; Kelly *et al.*, 1974). Finally, this study explains the very common observation that inhibitors of phosphodiesterases have a much more pronounced effect on stimulated than on resting cells. In stimulated cells the higher V_s/V_D ratio considerably increases the sensitivity of the system to small variations in phosphodiesterase activity (Fig. 4).

These examples show that the characteristics of phosphodiesterases are as important as those of adenylate cyclase in shaping the response of cells to signals acting on the latter enzyme. It is obvious that the influence of the kinetic properties of the disposal mechanism on the quantitative characteristics of cyclic AMP accumulation can be extended to other intracellular mediators or substrates.

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