CONSEQUENCES OF THE INTRACELLULAR DISTRIBUTION OF CYCLIC 3′,5′-NUCLEOTIDES PHOSPHODIESTERASES

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1. Introduction

Cyclic 3′,5′-adenosine monophosphate (cAMP) participates as second messenger of hormonal stimulation in a wide variety of intracellular stimulatory events [1]. The effects of such a stimulation are mediated by an increase of the intracellular concentration of cAMP. This concentration is regulated by cAMP synthesis from ATP by adenylate cyclase and cAMP degradation to 5′AMP by phosphodiesterases. Many hormones exert their effects by enhancing the activity of adenylate cyclase located in the plasma membrane [1]. As the destruction rate of cyclic AMP depends on the cAMP concentration, the system reaches at any time a steady state, i.e. the equalization of destruction and synthesis rates, by modifying the free cAMP level.

Intracellular cAMP is hydrolyzed at least by two types of phosphodiesterases characterized by different maximal activities, affinities for the substrate and subcellular localization (one enzyme is bound to the plasma membrane) [2–5]. Assuming that the two phosphodiesterases coexist in the same cell and that both exert their action on cAMP, the advantage for any cell of such a dual enzyme system is not obvious. One result of this situation may be to amplify the hormonal stimulation and thus to minimize the consumption of ATP needed by cAMP metabolism [6].

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Whereas in the cAMP system, both adenylate cyclase and one of the cAMP phosphodiesterase are located on the plasma membrane, in the cGMP sytem, guanylate cyclase and the phosphodiesterase appear to be soluble [4,7].

The purpose of this work was to examine the metabolic consequences of the different localizations of phosphodiesterases in the cell.

2. Description of the system

The role of intracellular cAMP is the propagation of a hormonal stimulus into the target cell, from the plasmatic membrane where adenylate cyclase is located, to the cAMP sites of action. The location of these sites depends in part on the cellular structure and on the physiological function of the tissue. For instance, the structure of the thyroid follicles affects the functional symmetry of the cell. Only a part of the plasmatic membrane namely the basal membrane may be attained by the stimulating hormone, and consequently, adenylate cyclase linked to the hormone receptors synthesizes the molecules of the cAMP mainly at this level. On the other hand, many effects of the hormonal stimulus take place at the proximity of the apical membrane. In this case, the molecules of cAMP must diffuse through the whole cytoplasm before transmitting the hormonal stimulus.

The activation of the system at any location depends on the spatial distribution of intracellular cAMP. The kinetics of the system are affected by the transport of cAMP into the cytoplasm.

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3. Theoretical resolution of the problem

3.1. *Spatial distribution of intracellular cAMP in the steady state*

The distribution of cAMP, thus the level of activation of cAMP effectors, is affected by the locations of the modulators (adenylate cyclase, phosphodiesterases) of the metabolism.

Let us consider a theoretical system which consists of a synthesis by A and a degradation by P of a component S. These two reactions and the possible transport of S take place in a closed cylinder (fig. 1).

Case 1

The molecules of A are concentrated at the base I, whereas the molecules of P are distributed homogeneously into the whole medium. Let x be any point of the medium not situated on the base I. At this point, S is degraded by P but is not synthesized because of the lack of A. Thus, there exists a flow of S to this point and consequently a gradient of concentration if transport occurs by diffusion. The concentration of S at the base II is lower than the one at the base I.

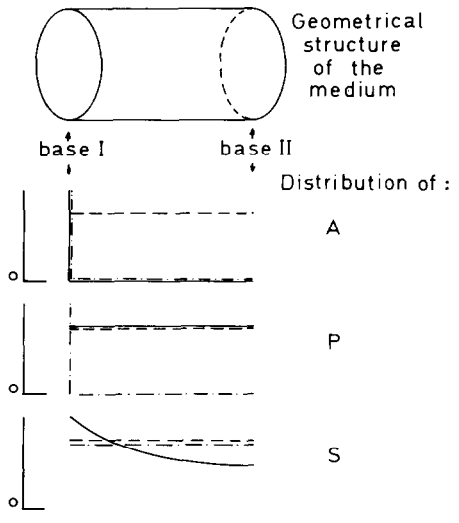

Fig. 1. Qualitative distribution of the component S and of its two modulators A and P. Case 1 (————); case 2a (. – . – . – .); case 2b (– – – –).

Case 2

a) if both A and P are located at the base I, S is not synthesized nor degraded at any inner point x. Since there is no diffusion flow of S in the steady state, the spatial distribution of S is homogeneous.
b) if both A and P are distributed homogeneously in the whole medium, then S is also distributed homogeneously because its synthesis and catabolism occur at the same place.

It clearly appears from this simple discussion that the gradient of S (e.g. cAMP) concentration depends on the respective locations of A (e.g. adenylate cyclase) and P (e.g. phosphodiesterase). Whatever the structure of the cell, the distribution of cAMP and consequently the distribution of the level of stimulation are homogeneous when the modulators are located at the same places (cases 2a, 2b).

Thus in the case of cAMP the location of one phosphodiesterase with high affinity for cAMP at the level of the plasma membrane and of adenylate cyclase would reduce the gradient of cAMP resulting from the activity of cytosol phosphodiesterase. Similarly, the presence of both guanylate cyclase and the corresponding phosphodiesterase in the cytosol insures the homogeneity of cellular cGMP.

3.2. *Kinetics of the system*

Some estimations of the apparent coefficient of diffusion D of molecules as nucleotides in the 'cell water' have been made (8–11]: we can accept the values 10^{-7} cm^2/sec and 10^{-6} cm^2/sec as limits of possible D. Assuming that the radius r of a thyroid cell is equal to $5 \cdot 10^{-4}$ cm, the characteristic diffusion time of a molecule of cAMP in such a cell would be between 0.5 sec and 5 sec. Thus, a molecule of cAMP can induce its effect at the apical membrane after a few seconds required for the transport between the two membranes. As the time for the thyroid cell to reach the half activated state is higher than 1 min [12], the delay due to the transport of cAMP should not appreciably affect the kinetics of activation.

The characteristics of the transmission by cAMP thus insures a rapid and uniform response of the whole system to the external stimulus. Nevertheless, the consumption of ATP needed by the cAMP metabolism for any stimulation level is higher if source and sink of cAMP were located at the membrane.

The numerical simulation has to show whether this consequence is important.

4. Transmission of hormonal stimulus in the cell

Digital simulation of the transmission of the hormonal signal (e.g. cAMP) in the thyroid cell has been made by a stochastic method, a generalization to the one dimension random walk method [13,14] on digital computers (Wang 2200 and CDC 6500). In this method the chemical reactions take place in a number of discrete channels, in each of which the cAMP concentration is homogeneous; diffusion of the small molecules (e.g. cAMP) is simulated as the passage of these molecules between adjacent channels.

Numerical data as the elements of the sytem (table 1) have been obtained from results of our laboratory on thyroid [15] and from data on liver [4,16]. The conclusions drawn from this simulation are very general and do not depend on the absolute values used.

Fig. 2 shows the influence of the localization of the phosphodiesterase in the basal membrane. The homogeneity of the cAMP distribution in the steady state may be characterized by the ratio $R = \frac{B - A}{M}$ where B,A and M are respectively the highest (basal membrane), the lowest (apical membrane) and the mean concentration of cAMP. The relative range R which measures the heterogeneity of cAMP distribution, is given for different cases in table 2. It appears clearly that the presence of a membrane bound phosphodiesterase greatly minimizes the heterogeneity of cAMP concentration around the mean concentration.

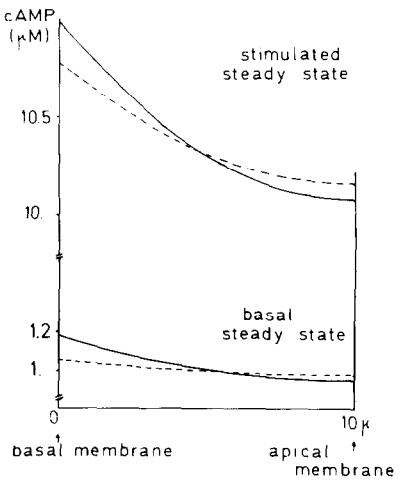

Fig. 2. (———); Theoretical cAMP distribution for the unrealistic case: two soluble phosphodiesterases. Mean cAMP level: basal 1.03 10^{-6} M; stimulated: 1.04 10^{-5} M. (– – –): Theoretical cAMP distribution for the realistic case: one membrane bound phosphodiesterase and one soluble phosphodiesterase. Mean cAMP level: basal 1.01 10^{-6} M; stimulated: 1.04 10^{-5} M.

We have considered here the simplest hypothesis that the whole amount of cAMP present in the cell participates in its action, especially in the activation of protein kinase. The hypothesis of sequestrating structures for cAMP is unnecessary [17] and not supported by experimental evidence in several systems [18–20]. Thus, the concentrations of free cAMP deduced from the simulation must reflect the level of activation.

The numerical simulation shows that the transport of cAMP does not affect the rate of activation of the

Table 1

Adenylate cyclase:	– basal activity = 2.8 10^{-6} M/min – stimulation factor by TSH = 4
Phosphodiesterase I:	– soluble enzyme – $K_m = 4.10^{-5}$ M – $V_m = 3.7\ 10^{-5}$ M/min
Phosphodiesterase II:	– membrane bound enzyme – $K_m = 10^{-6}$ M – $V_m = 3.7\ 10^{-6}$ M/min

Table 2

Realistic case (membrane bound + soluble phosphodiesterases)

	Basal steady state		Stimulated steady state	
D (cm²/sec)	10^{-6}	10^{-7}	10^{-6}	10^{-7}
R	.007	.073	.005	.060
Unrealistic case (two soluble phosphodiesterases) (given for comparison)				
R	.023	.221	.009	.088

cell. Indeed, let δt^+ (δt^-) be the time needed for the transition from the instimulated (stimulated) steady state to the half activated state for cAMP. Without taking diffusion into account, the time transitions are equal to $\delta t^+ = 59$ sec and $\delta t^- = 45$ sec. If the 'diffusion reaction' coupled system were simulated, the time transitions do not vary significantly (about 1%). The simulation also draws that for a given level of cAMP concentration at the apex, adenylate cyclase activity is lower when one phosphodiesterase is bound at the basal membrane, i.e. to achieve a definite level of stimulation is more economical for the cell even though more cAMP is hydrolyzed at the basal membrane. However in terms of adenylate cyclase activity (i.e. ATP consumption) required to elicit a given stimulation these advantages are small (about 1%).

5. Conclusion

The present study shows that the presence at the same location in the cell of the enzymatic systems responsible for the synthesis and degradation of a metabolite or signal (in this case cAMP) ensures a more homogeneous distribution of this agent in the cell. In the case of the thyroid cAMP system, it has been shown that diffusion is rather fast and thus plays little role in the kinetics of cAMP action on the cell.

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