

Adenylate cyclase inhibition by hormones

The Mg^{2+} hypothesis

Joel Bockaert and Michele Sebben-Perez

Centre CNRS, INSERM de Pharmacologie, Endocrinologie, Rue de la Cardonille, BP 5055, 34033 Montpellier Cédex, France

Received 22 July 1983

In washed anterior pituitary membranes, there is enough GTP to occupy N_s and therefore to obtain activation of adenylate cyclase by vasointestinal peptide. GTP concentrations needed to obtain adenylate cyclase inhibition by dopamine (above 5×10^{-7} M) stimulate the adenylate cyclase. The dopamine effect is a blockade of this stimulation. We propose that at least in this system, N_i does not inhibit but stimulates the adenylate cyclase and that inhibitory hormones block this stimulation. We also demonstrate in several adenylate cyclase systems that hormones produced adenylate cyclase inhibition by lowering their Mg affinity. A general model for adenylate cyclase activation and inhibition is proposed.

Adenylate cyclase inhibition

Magnesium

Guanosine-triphosphate

1. INTRODUCTION

Recent and important progress has been made in the understanding of the molecular mechanisms by which hormones and neurotransmitters stimulate the adenylate cyclase. At least 3 proteins are involved: a receptor, a catalyst and a nucleotide-binding protein called G/F, N_s or G [1–5]. N_s is composed of two subunits: a 35 kDa subunit and a 42–45 kDa protein [4,6–7]. Activation of N_s is the rate-limiting step in adenylate activation and is probably the result of a Mg^{2+} -GTP-dependent dissociation of the two subunits [3,7]. The GTP site is localized on the 45 kDa protein [7]. Once dissociated, the 45 kDa subunit interacts with the catalytic unit and activates the enzyme. Stimulatory hormones enhance the dissociation between N_s subunits by increasing the Mg^{2+} affinity of the system [3]. Authors in [3] therefore considered the hormone occupied receptor as a 'Mg²⁺ switch' system [3].

As far as inhibitory hormones are concerned, much less is known. It is clear that higher GTP

concentrations are necessary to obtain hormone-dependent adenylate cyclase inhibition than hormone-dependent adenylate cyclase stimulation [2]. In [2] it was first proposed that the GTP-binding protein involved in adenylate cyclase inhibition is different from the GTP-binding protein involved in adenylate cyclase stimulation and was called N_i [2]. Recent studies suggested that N_i is composed of two subunits = a 35 kDa subunit (probably identical to the 35 kDa subunit of N_s) and a 39–41 kDa subunit which can be specifically ADP-ribosylated by the toxin of *Bordetella pertussis* [8,9].

So far, the general hypothesis is that N_i , once occupied by GTP, is able to interact with the catalytic unit to trigger adenylate cyclase inhibition. Here we present evidence suggesting the paradox that N_i can, at least in some systems, stimulate the catalytic unit of the adenylate cyclase and that this stimulation is inhibited by receptors occupied by inhibitory hormones. Furthermore, we demonstrated that this inhibition is due to a decrease in the Mg^{2+} affinity of the system.

2. MATERIALS AND METHODS

2.1. Membrane preparation from anterior pituitary lobe

Female Wistar rats (200–250 g) were killed by decapitation. Anterior pituitary lobes from 6 rats were homogenized in a glass-teflon potter homogenizer in 1 ml of 1 mM Tris–maleate buffer (pH 7.2), 1 mM EGTA and 300 mM sucrose (TES medium). Two ml of the same medium without sucrose was added. Membranes were pelleted by centrifugation at $10000 \times g$ and resuspended in 1.5 ml TES.

2.2. Adenylate cyclase assay

10 μ l of membranes were added to the assay (50 μ l) which contained: 50 mM Tris–maleate (pH 7.2), 1 mM cAMP, 0.15 mM ATP, 0.01 mM GTP, 10 mM theophylline, 5 mM creatine phosphate, 0.2 mg/ml creatine–kinase, 1–2 μ Ci [α - 32 P]ATP and 0.001 μ Ci [3 H]cAMP. Mg^{2+} concentrations were added as indicated. Incubation was 10 min at 30°C. Cyclic [32 P] AMP was purified as in [10].

2.3. Computer analysis

The data were analysed by a non-linear least squares curve fitting procedure using a 'Minuit' routine for function analysis described in [11]. The formula used was:

$$V = V_1 \frac{S}{S + K_{m,1}} + V_2 \frac{S}{S + K_{m,2}}$$

in which V is the total adenylate cyclase activity, S the Mg^{2+} concentration, V_1 and V_2 the respective maximal activities of the components of the complex assumed to be responsible for activity at low Mg^{2+} concentrations ('high Mg^{2+} affinity component') and high Mg^{2+} concentrations ('low Mg^{2+} affinity component'). $K_{m,1}$ and $K_{m,2}$ are apparent K_m -values of the high and low Mg^{2+} affinity components. The suitability of the fit was evaluated as proposed in [12].

3. RESULTS AND DISCUSSION

In washed membranes from anterior pituitary, there is enough GTP to occupy N_S and therefore to obtain adenylate cyclase activation by vasointesti-

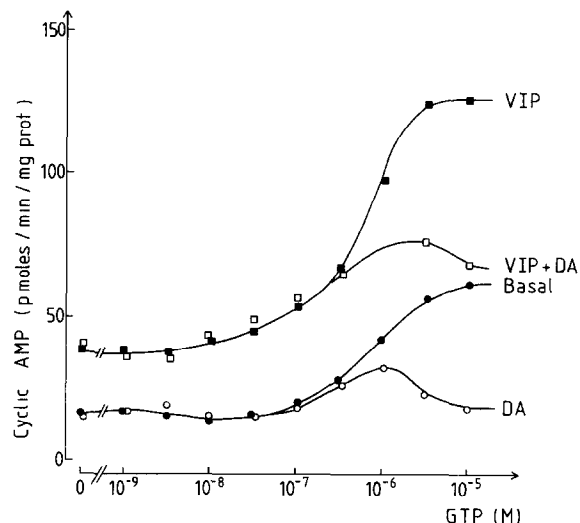


Fig.1. GTP dependence of basal, VIP adenylate cyclase activities in the absence (●, ■) and presence of DA (○, □). Mg^{2+} , VIP and DA concentrations were 1.5 mM, 10^{-7} and 10^{-5} M, respectively.

nal peptide (VIP) (fig.1). The stimulatory action of VIP was not significantly modified when GTP concentrations were increased. On the contrary, in the absence of added GTP, dopamine (DA) was unable to inhibit the enzyme (fig.1). The inhibition was only apparent for GTP concentrations above 5×10^{-7} M and appeared to be due to a blockade of basal adenylate cyclase stimulation produced by μ M GTP concentrations. Therefore, the GTP-binding protein involved in this stimulation has the following characteristics:

- (i) It is not required to obtain adenylate cyclase stimulation by the stimulatory hormone.
- (ii) It is required to obtain adenylate cyclase inhibition by the inhibitory hormone.

Therefore, we propose that at least in this system, N_i does not inhibit, but stimulates the adenylate cyclase system. DA triggers its inhibition by blocking this stimulation (fig.1).

Another interesting observation made on this system was that this blockade was due to a change in the Mg^{2+} affinity of the system (fig.2). In the absence of DA, and in the presence of GTP, the Mg^{2+} dose activation curve could be fitted according to a simple Michaelis–Menten kinetic ($K_m = 0.43$ mM) (fig.2). Fig.2 shows that DA inhibited the enzyme by decreasing the Mg^{2+} affinity without modifying the V_{max} . Computer analysis of

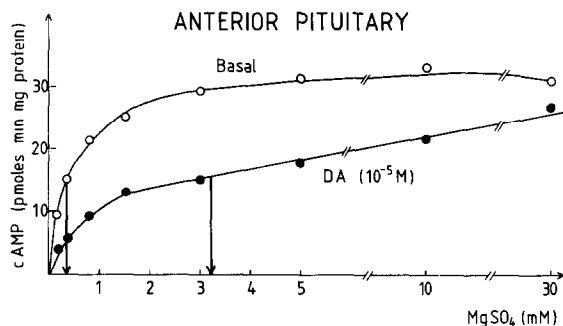


Fig.2. Basal and dopamine-inhibited adenylate cyclase as the function of Mg^{2+} . Computer analysis indicated that under control conditions, 100% of the enzyme had an apparent Mg^{2+} affinity of 0.43 mM (\circ). In the presence of DA (10^{-5} M) (\bullet) the curve could be fitted by the sum of 2 components. A high Mg^{2+} affinity component ($K_m = 0.5$ mM; $V_{max} = 36\%$) and a low Mg^{2+} affinity component ($K_m = 14.1$ mM; $V_{max} = 64\%$) ($F = 44$; $p < 0.01$ as compared to a single component).

the Mg^{2+} activation curve in the presence of DA revealed that two components were present, one representing 36% of the total activity and having the initial high affinity for Mg^{2+} (0.5 mM), the other representing 64% and having a lower affinity (14.1 mM). This result is interesting since it suggests that inhibitory hormones could act by a more symmetrical mechanism of action than the one demonstrated for stimulatory hormones.

In several other systems, human platelets, liver membranes and adipocytes, we also demonstrated that hormones or neurotransmitters inhibit the enzyme by an identical ' Mg^{2+} switch' process. In human platelet, computer analysis revealed that only two states of the enzyme exist. Increasing hormonal concentrations progressively switch the system from a high to a low affinity state (Bockaert et al., in preparation). Fig.3 proposed a speculative and probably oversimplified model of adenylate cyclase activation and inhibition. In the absence of GTP and Mg^{2+} the adenylate cyclase activity is low. The 3 subunits which constitute N_s and N_i formed a complex devoid of stimulatory or inhibitory properties (I). In the presence of Mg^{2+} and low GTP concentrations (nM) the dissociation of 35 kDa and 45 kDa occurred as proposed in [3,7].

The 45 kDa occupied by GTP stimulated the

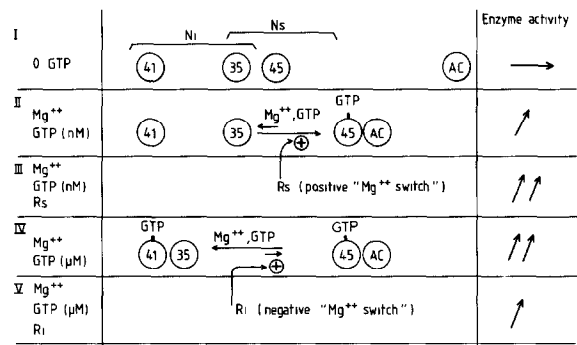


Fig.3. Hypothetical model for adenylate cyclase activation and inhibition. For explanations see text. N_i and N_s are the inhibitory and stimulatory binding proteins, respectively. R_s and R_i are the hormone receptor complexes formed in the presence of stimulatory and inhibitory hormones, respectively.

catalytic unit (II). If a stimulating hormone is added, there is an increase in the Mg^{2+} affinity of the system leading to an enhancement of the N_s subunits dissociation. The adenylate cyclase activity is further increased (III). When GTP concentrations reached the μ M ranges the 41 kDa protein occupied by GTP interacted with the 35 kDa protein. This process is GTP- Mg^{2+} -dependent and enhances the dissociation between the 35 kDa and 45 kDa subunits. The free 45 kDa protein stimulates the catalytic unit (IV). If an inhibitory hormone is added, there is a decrease in the Mg^{2+} affinity of the system; the 41–35 kDa complex dissociates, leading to a reassociation between the 35 and 45 kDa subunits. This reassociation produces a reversal of the adenylate cyclase activation (V). A variation of this model could be that the 41 kDa protein, when occupied by GTP stimulates directly the adenylate cyclase. The decrease in Mg^{2+} affinity produced by inhibitory hormones leads to a reassociation between the 41 kDa and 35 kDa subunits giving an inactive complex. More work with purified N_i and N_s subunits will discriminate between these different possibilities.

ACKNOWLEDGEMENTS

We would like to thank Dr L. Birnbaumer for fruitful discussions and G. Vassent for computer analysis.

REFERENCES

- [1] Pfeuffer, T. (1979) FEBS Lett. 101, 85–89.
- [2] Rodbell, M. (1980) Nature 284, 17–22.
- [3] Iyengar, R. and Birnbaumer, L. (1982) Proc. Natl. Acad. Sci. USA 79, 5179–5183.
- [4] Northrup, J.K., Sternweins, P.C., Smigel, M.D.M., Schleifer, L.S., Ross, E.M. and Gilman, A.G. (1980) Proc. Natl. Acad. Sci. USA 77, 6516–6520.
- [5] Ross, E.M. and Gilman, A.G. (1977) J. Biol. Chem. 252, 6966–6969.
- [6] Cassel, D. and Pfeuffer, T. (1978) Proc. Natl. Acad. Sci. USA 75, 2669–2673.
- [7] Northrup, J.K., Smigel, M.D. and Gilman, A. (1982) J. Biol. Chem. 257, 11416–11423.
- [8] Hildebrandt, J.D., Sekura, R.D., Codina, J., Iyengar, R., Manclark, C.R. and Birnbaumer, L. (1983) Nature 302, 706–709.
- [9] Bobock, G.M., Katada, T., Northrup, J.K., Hewlett, E.L. and Gilman, A.G. (1983) J. Biol. Chem. 258, 2072–2075.
- [10] Bockaert, J., Hunzicker-Dunn, M. and Birnbaumer, L. (1976) J. Biol. Chem. 251, 2653–2663.
- [11] James, F. and Roos, M. (1975) Computer Physics Commun. 10, 343–367.
- [12] De Lean, A., Stadel, J.M. and Lefkowitz, R.J. (1980) J. Biol. Chem. 255, 108–117.