PROMOTION BY Mg^{2+} OF GUANOSINE 5'-(β,γ-IMIDO)TRIPHOSPHATE ACTIVATION OF ADENYLATE CYCLASE IN RAT LUNG AND HEART MEMBRANES

Pierre CHATELAIN+, Patrick ROBBERECHT, Anh NGUYEN HUU+ and Jean CHRISTOPHE*

Department of Biochemistry and Nutrition, Medical School, Université Libre de Bruxelles, Boulevard of Waterloo 115, B-1000 Brussels and *+Continental Pharma, Research Laboratories, Parc Scientifique, rue Granbonpré 11, B-1348 Mont-Saint-Guibert, Belgium*

Received 24 March 1982

1. Introduction

The interaction of the components of adenylate cyclase (ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1) located at the inner face of plasma membranes is regulated, inter alia, by guanine nucleotides [1] and Mg^{2+} [2]. The role of guanine nucleotides has been extensively investigated with non-hydrolyzable analogues, by GTPase inhibition after cholera toxin pretreatment, and by solubilization and reconstitution experiments [1,3,4].

Much less is known on the multiple role of Mg^{2+} apart from its contribution to the substrate ATP-Mg. Kinetics of adenylate cyclase activation are usually linear except upon exposure to non-hydrolyzable guanine nucleotide analogues for which a lag period in cyclic AMP accumulation curve is generally observed. The modulation of this lag period by Mg^{2+}, first described in rat reticulocytes [5], has been observed in other membranes including those from rat liver, S49 murine lymphoma cells, and rabbit corpus luteum [6,7]. These data, plus the Mg^{2+} requirement for persistent Gpp[NH]p activation of heart adenylate cyclase [8], and the blockade of Gpp[NH]p activation of liver adenylate cyclase by EDTA [9], allows to conclude that Mg^{2+} acts on an allosteric site in guanine nucleotide regulatory protein(s).

Our original finding that there is no lag period for Gpp[NH]p activation of rat cardiac adenylate cyclase under standard conditions with 5 mM Mg^{2+} [10,11] prompted us to further analyze and compare the effects of Mg^{2+} in rat heart and lung membranes. We conclude that the allosteric stimulatory role of Mg^{2+} is similar but variously expressed among mammalian tissues.

Abbreviation: Gpp[NH]p, guanosine 5'-(β,γ-imido)triphosphate

* To whom correspondence should be addressed

2. Materials and methods

Wistar albino male rats (200–250 g) with free access to standard food and water were sacrificed by decapitation. Heart and lungs were dissected out, rinsed at room temperature with isotonic NaCl, and minced with scissors. All subsequent operations were performed at 4°C.

A crude cardiac particulate fraction was prepared with minor modifications of the procedure in [12]. The cardiac tissue was homogenized (5% (w/v) homogenate) in a 20 mM Tris–HCl, 2 mM dithioerythreitol, 5 mM $MgCl_2$ (pH 7.5) buffer. After filtration through 2 layers of medical gauze, the homogenate was centrifuged at 520 × *g* for 10 min. The pellet was washed once with the same buffer and resuspended in 20 mM Tris–HCl, 0.25 M sucrose (pH 7.5) buffer. An equal volume of the same buffer enriched with 2.5 M KCl was added dropwise. The suspension was stirred continuously for 2 h at 4°C, then centrifuged at 37 000 × *g* for 10 min. The pellet was resuspended in a 20 mM Tris–HCl, 2 mM dithioerythreitol, 0.25 M sucrose (pH 7.5) buffer, washed 3 times in this buffer by centrifugation at 31 000 × *g* for 5 min and resuspended in a volume of the same buffer allowing a final concentration of 6 mg protein/ml.

Lungs were homogenized (5% (w/v) homogenate) in a 20 mM Tris–HCl, 2 mM dithioerythreitol, 0.25 M sucrose (pH 7.5) buffer. After filtration through 2 layers of medical gauze, the homogenate was centri-

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$02.75

fuged at 31 000 X *g* for 10 min. The pellet was resuspended in the same buffer at 10 mg protein/ml final conc.

Adenylate cyclase activity was determined by the conversion of [α-^{32}P]ATP into cyclic [^{32}P]AMP [13]. The assay medium contained the following final concentrations: 0.5 mM [α-^{32}P]ATP, 0.5 mM EGTA, 1 mM cyclic AMP, 1 mM theophylline, 30 mM Tris–HCl, and an ATP-regenerating system which consisted of 10 mM phospho(enol)pyruvate and pyruvate kinase (30 μg/ml). The pH was adjusted to 7.4. The kinetics of adenylate cyclase activity were determined as follows: each glass tube contained 0.60 ml standard assay medium equilibrated at 37°C. The incubation was initiated by adding 0.12 ml of the membrane suspension equilibrated at the desired [Mg^{2+}]. At fixed intervals (1 or 2 min), 0.05 ml aliquots of the incubation medium were removed and added to 0.50 ml of a 0.5% SDS solution containing 1.5 mM ATP, 0.5 mM cyclic AMP and cyclic [8-^{3}H]AMP (20 000 cpm to determine nucleotide recovery). Cyclic AMP was separated from ATP by 2 successive chromatographies on Dowex AGI X 8 and neutral alumina [13].

Phospho(enol)pyruvate, pyruvate kinase, ATP (sodium salt, grade I). GTP, cyclic AMP, and D,L-isoproterenol were purchased from Sigma Chemical Co. (St Louis, MO). Gpp[NH]p was obtained from Boehringer (Mannheim). Cyclic [8-^{3}H]AMP and [α-^{32}P]ATP, obtained from the Radiochemical Centre (Amersham, Bucks), had spec. act. 27 Ci/mmol and 10–20 Ci/mmol, respectively.

3. Results

In the presence of a maximally activating Gpp[NH]p concentration, the rate of cyclic AMP production in both heart (fig.1) and lung (fig.2) membranes depended on [Mg^{2+}]. At a low (0.5 mM) Mg^{2+} concentration, a lag period was obvious. When the divalent ion concentration was progressively increased above ATP concentration, the lag period decreased and the enzyme activity increased. In heart, the rate of activation was both maximal and linear at 4 mM Mg^{2+} (fig.1) so that higher Mg^{2+} levels (at 6 mM (fig.1), 8 mM, 20 mM and 30 mM (not shown)) exerted no added effect. The low basal activities in the absence of Gpp[NH]p were linear at all [Mg^{2+}] tested (not shown).

The effects observed on lung adenylate cyclase activity with increasing [Mg^{2+}] (fig.2) were qualitatively similar but the lung enzyme was less sensitive

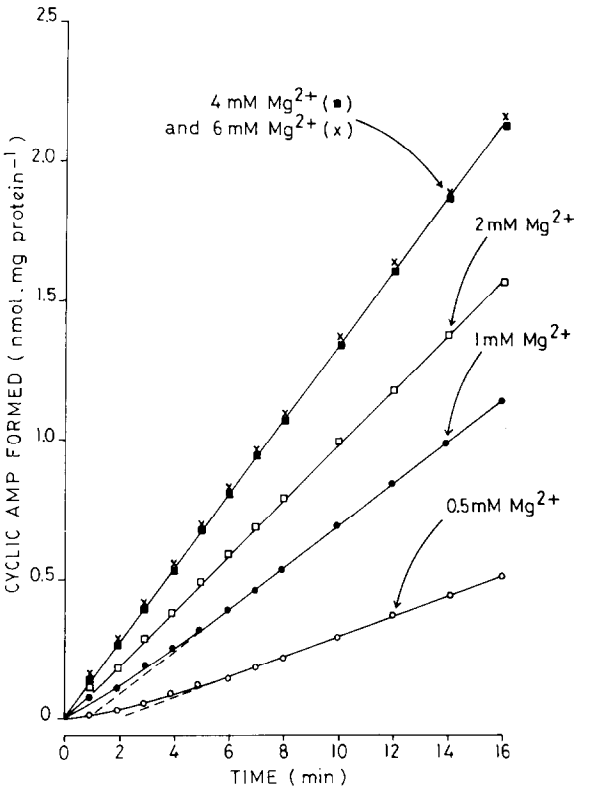

Fig.1. Dependence on [Mg^{2+}] of the time course of activation of rat cardiac adenylate cyclase by 10^{-4} M Gpp[NH]p; Mg^{2+} levels indicated were the total [Mg^{2+}] in the incubation medium. For medium composition and incubation conditions, see section 2. The apparent lag period is obtained by extrapolation (– – –). This experiment was representative of 4 others.

to Mg^{2+} than the heart system. At any given [Mg^{2+}] the lag period was, indeed, more important in lung than in heart membranes (the lag period at 0.5 mM Mg^{2+} in lung was, for instance, twice the lag period in heart). At 4–6 mM Mg^{2+}, that are commonly used in adenylate cyclase assays, the lag period was still apparent in lung while the rate of activation was linear in heart.

As shown in fig.3,4, lag periods were also observed at 0.5 mM Mg^{2+} when adenylate cyclase activity was stimulated by NaF in heart and lung membranes, and by GTP in lung membranes. (No significant activation by GTP could be observed in heart as in [10,14].) With NaF activation, increasing the [Mg^{2+}] increased the enzyme activity and linearized the formation of cyclic AMP as a function of time (fig.3,4). Finally, 0.1 mM D,L-isoproterenol was also able to linearize the time course of Gpp[NH]p activation at all Mg^{2+} levels (fig.3,4, not shown).

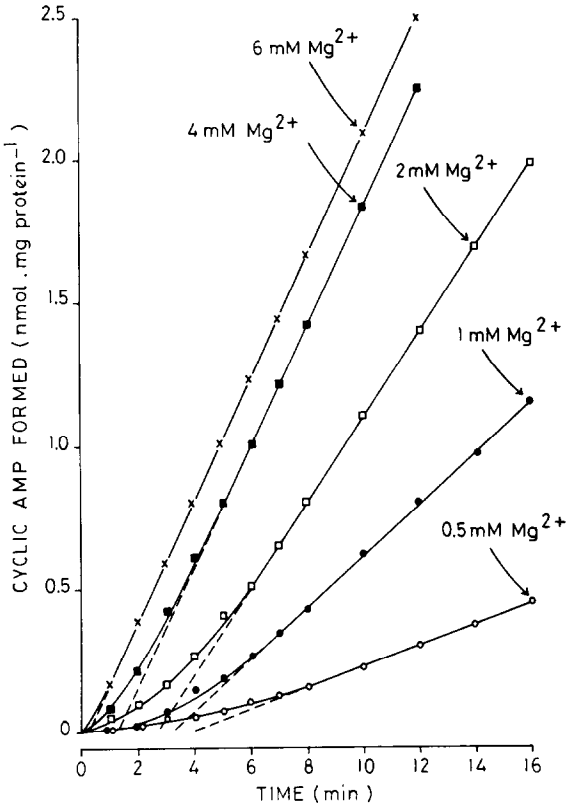

Fig.2. Dependence on $[Mg^{2+}]$ of the time course of activation of rat lung adenylate cyclase by 10^{-4} M Gpp[NH]p. For details, see legend for fig.1. This experiment was representative of 4 others.

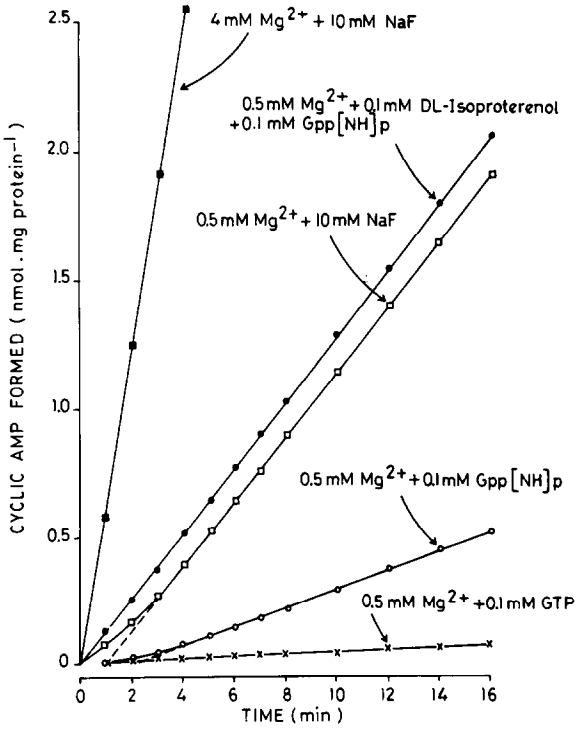

Fig.3. Time course of activation of rat cardiac adenylate cyclase by 10^{-4} M GTP (X), 10^{-4} M Gpp[NH]p (○), 10^{-4} M Gpp[NH]p + 10^{-4} M D,L-isoproterenol (●), and 10^{-2} M NaF (□,■). Mg^{2+} levels refer to total $[Mg^{2+}]$ concentrations in the incubation medium. For details, see legend for fig.1. This experiment was representative of 4 others.

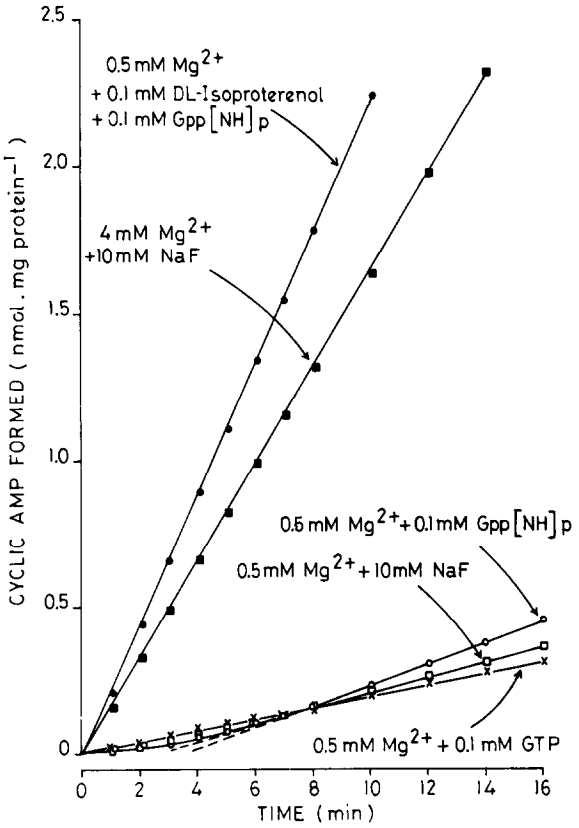

Fig.4. Time course of activation of rat lung adenylate cyclase by 10^{-4} M GTP (X), 10^{-4} M Gpp[NH]p (○), 10^{-4} M Gpp[NH]p + 10^{-4} M D,L-isoproterenol (●), and 10^{-2} M NaF (□,■). Mg^{2+} levels refer to total $[Mg^{2+}]$ in the incubation medium. For details, see legend for fig.1. This experiment was representative of 4 others.

4. Discussion

In line with results on membranes from rat liver, S49 murine lymphoma cells, and rabbit corpus luteum [6,7], these data showed that the lag period of adenylate cyclase activation by Gpp[NH]p was modulated by [Mg^{2+}] in both heart and lung membranes from rat. This indicates that a lag period for Gpp[NH]p activation is a general phenomenon in mammalian adenylate cyclase systems and contrasts with the situation in turkey erythrocyte where Mg^{2+} is unable to modify this parameter [6].

Quantitative differences were obvious when comparing heart and lung membranes (fig.1,2), that were not due to the mode of preparation of rat cardiac membranes, as identical results were obtained with a crude particulate heart fraction untreated with KCl. These differences might, conceivably, reflect distinct intrinsic properties of the guanine nucleotide regulatory protein(s) or the presence of other controlling factor(s) such as the lipidic environment.

Of particular interest was the fact that a lag period was observed, at 0.5 mM Mg^{2+}, with GTP activation in lung membranes (fig.4) and also with NaF activation in both heart and lung membranes (fig.3,4). This confirms [6,7] that the lag period depends on several factors, including the chemical nature of the nucleotide activator and a stimulatory effect of Mg^{2+} on the activation of guanine nucleotide regulatory protein(s).

In the presence of D,L-isoproterenol and Gpp[NH]p, the time course of cyclic AMP formation was linear at all [Mg^{2+}] tested (fig.3,4, not shown). At least two explanations may account for the disappearance of the lag period due to D,L-isoproterenol:

(1) The time period required for endogenous nucleotides to dissociate from guanine nucleotide regulatory protein(s) may be responsible for the lag period; its shortening could result from the D,L-isoproterenol-stimulated release and/or exchange of endogenous nucleotides [15,16];

(2) The lag period might be due to a slow isomerization between two activity states of the system with the hormone increasing the rate of isomerization to the active state [17,18].

Conceivably, a hormone and Mg^{2+}, when used in combination, may simply exert additive effects on the reduction of the lag period; alternatively, the acceleration of the isomerization process induced by the hormone may decrease the allosteric Mg^{2+} requirements.

Acknowledgements

We thank Dr R. Iyengar for making his results available prior to publication. Aided by grant 3317 from IRSIA (Belgium) and by grant 3.4504.81 from the Fonds de la Recherche Scientifique Médicale (Belgium).

References

[1] Rodbell, M. (1980) Nature 284, 17–22.
[2] Birnbaumer, L., Pohl, S. L. and Rodbell, M. (1969) J. Biol. Chem. 244, 3468–3476.
[3] Cassel, D. and Pfeuffer, T. (1978) Proc. Natl. Acad. Sci. USA 75, 2669–2673.
[4] Citri, Y. and Schramm, M. (1980) Nature 287, 297–300.
[5] Wiemer, G., Kaiser, G. and Palm, D. (1978) Naunyn-Schmiedeberg's Arch. Pharmacol. 303, 145–152.
[6] Iyengar, R. and Birnbaumer, L. (1981) J. Biol. Chem. 256, 11036–11041.
[7] Iyengar, R. (1981) J. Biol. Chem. 256, 11042–11050.
[8] Alvarez, R. and Bruno, J. J. (1977) Proc. Natl. Acad. Sci. USA 74, 92–95.
[9] Lad, P. M., Welton, A. F. and Rodbell, M. (1977) J. Biol. Chem. 252, 5942–5946.
[10] Chatelain, P., Robberecht, P., De Neef, P., Claeys, M. and Christophe, J. (1979) FEBS Lett. 107, 86–90.
[11] Chatelain, P., Robberecht, P., De Neef, P., Camus, J. C., Poloczek, P. and Christophe, J. (1981) Pflügers Arch. 390, 10–16.
[12] Snyder, F. F. and Drummond, G. I. (1978) Arch. Biochem. Biophys. 185, 116–125.
[13] Salomon, Y., Londos, C. and Rodbell, M. (1974) Anal. Biochem. 58, 541–548.
[14] Hegstrand, L. R., Minneman, K. P. and Molinoff, P. B. (1979) J. Pharm. Exp. Ther. 210, 215–221.
[15] Blume, A. J. and Foster, C. J. (1976) J. Biol. Chem. 251, 3399–3404.
[16] Cassel, D. and Selinger, Z. (1977) Proc. Natl. Acad. Sci. USA 74, 3307–3311.
[17] Birnbaumer, L., Swartz, T. L., Abramowitz, J., Mintz, P. W. and Iyengar, R. (1980) J. Biol. Chem. 255, 3542–3551.
[18] Svoboda, M., Lambert, M. and Christophe, J. (1981) Biochim. Biophys. Acta 675, 46–61.