

CALCIUM ION: A MODULATOR OF PAROTID ADENYLATE CYCLASE ACTIVITY

Douglas J. FRANKS, Lee S. PERRIN and Daniel MALAMUD

Shriners Burns Institute, Massachusetts General Hospital, Boston, Massachusetts 02114, U.S.A.

and

Department of Surgery, Harvard Medical School, Cambridge, Massachusetts, U.S.A.

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1. Introduction

Adenylate cyclase activity *in vitro* can be stimulated by hormones, prostaglandins, and NaF. NaF activates the enzyme from all tissues by a mechanism that is still not understood [1] and the E series of prostaglandins can either stimulate, inhibit, or have no effect on adenylate cyclase activity [2,3]. Attempts to purify adenylate cyclase from membranes by solubilization usually result in the loss of stimulation by hormones and prostaglandins, the solubilized enzyme being activated only by NaF [4].

We have been attempting to purify adenylate cyclase from mouse parotid gland, a tissue that has high basal adenylate cyclase activity [5]. In the course of these studies we observed that both the particulate and the solubilized enzyme exhibited a dependence on Ca^{2+} ion. This property, along with activation by NaF, may be of value in elucidating the mechanism of action of adenylate cyclase, since the effects appear to be manifest directly on the catalytic unit of the enzyme.

2. Materials and methods

Adenylate cyclase activity was determined by measuring the conversion of ATP $\alpha^{32}\text{P}$ into cAMP ^{32}P during 10 min incubation at 37°C in a reaction mixture containing 25 mM Tris-HCl (pH 7.5), 6 mM MgCl_2 , 1 mM EDTA, 3 mM mercaptoethanol, 5 mM cAMP, ATP $\alpha^{32}\text{P}$ (1mM and 10-20 dpm/pmole),

0.0075% bovine serum albumin, an ATP regeneration system consisting of 5.4 mM phosphoenolpyruvate and 2.5 units pyruvate kinase, and 0.1–0.2 mg of enzyme protein in a total vol of 0.1 ml.

Reactions were terminated by the addition of 0.1 ml 40 mM Tris-HCl (pH 7.5), 40 mM ATP and 12.5 mM cAMP ^3H (10 000 cpm). cAMP was isolated on columns of neutral alumina [6] with a recovery of 90–95%. The reaction rate was linear with time and with protein concentration under the assay conditions used.

3. Results

Homogenates of parotid gland demonstrated high basal adenylate cyclase activity that was stimulated 2-fold by 0.1 mM isoproterenol (IPR) and 4-fold by 10 mM NaF (fig. 1.). Enzyme activity was dependent on Mg^{2+} in a manner characteristic of adenylate cyclase from other tissues [7,8], demonstrating optimal activity when the Mg^{2+} concentration was 5 times that of ATP.

Ethylene glycol bis (β aminoethyl ether) NN'-tetraacetic acid (EGTA), a chelator of calcium, progressively inhibited enzyme activity at concentrations ranging from 0.05–1.0 mM (fig. 1.). Inhibition was maximal at 0.5 mM EGTA; basal, IPR and NaF stimulated activities were inhibited to the same extent. The inhibition could be reversed by the addition of CaCl_2 ; activity returned to normal when the calcium ion concentration was equimolar with that of EGTA (fig.

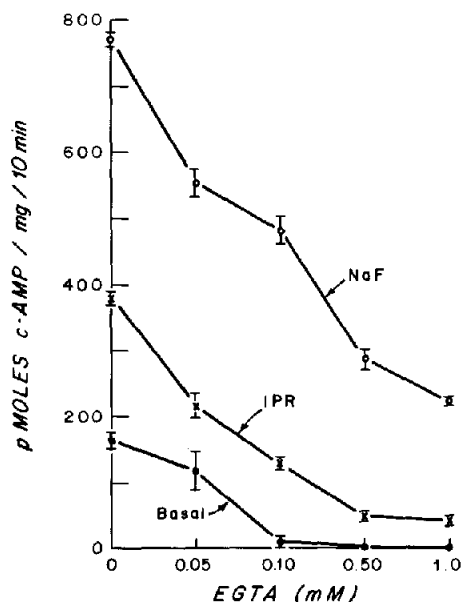


Fig. 1. Effect of increasing concentrations of EGTA on adenylate cyclase activity of mouse parotid homogenate. NaF concentration was 10 mM and IPR was 0.1 mM. Assays were carried out using 100 μ g of protein. Each point represents the mean \pm S.E. of at least 3 determinations.

2A). Since EGTA is not totally specific for calcium it is possible that the addition of calcium could displace some other divalent cation that is present at very low concentrations. However, because there is a high concentration of calcium in the parotid [9], this ion seems the most likely candidate. Furthermore the response of adenylate cyclase to calcium is biphasic (fig. 2B); 0.1 mM CaCl_2 caused a statistically significant ($p < 0.1$) increase in both basal and stimulated enzyme activity and pronounced inhibition above 0.5 mM.

Calcium is not required for hormonal activation of the enzyme as in other tissues [10], since IPR stimulates parotid adenylate cyclase in the presence of EGTA. The calcium dependence of parotid adenylate cyclase seems to involve a requirement for calcium at the catalytic site of the enzyme. This was confirmed by homogenizing parotid tissue in Lubrol-PX, a non-ionic detergent previously used by Levey to solubilize myocardial adenylate cyclase [4]. Enzyme solubilized by this procedure demonstrated high basal activity and was stimulated by NaF but not by IPR. The solubilized

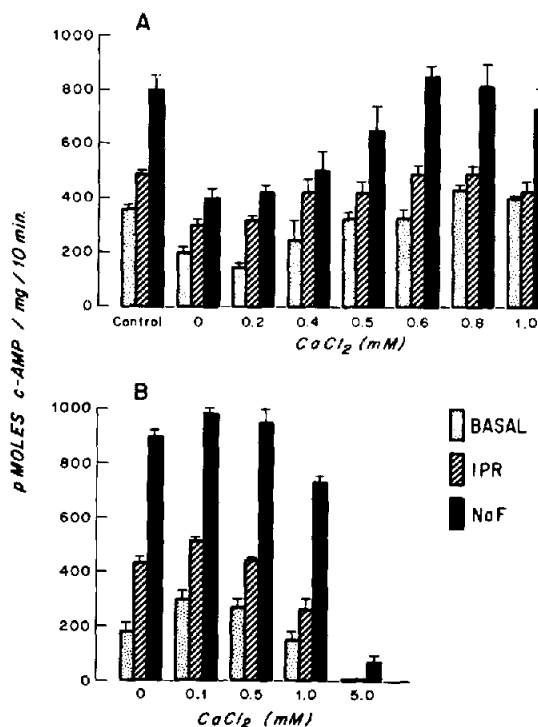


Fig. 2. (A) Effect of the addition of CaCl_2 to adenylate cyclase assayed in the presence of 0.5 mM EGTA. Control refers to determinations made in the absence of EGTA and CaCl_2 . (B) Activity of parotid adenylate cyclase in the presence of increasing concentrations of CaCl_2 . The concentrations of NaF and IPR were the same as in fig. 1.

adenylate cyclase showed the same calcium dependence as did the wholly particulate enzyme (table 1), but it was not stimulated by low concentrations of calcium ion alone suggesting a possible additional role for calcium as a coupler between hormone receptor and catalytic unit.

4. Discussion

Inhibition of adenylate cyclase by calcium has been demonstrated in preparations from a number of tissues [11], while stimulation by calcium has been reported only for the brain enzyme [12]. We have found that EGTA did not affect adenylate cyclase from mouse liver and kidney, while enzyme from whole mouse brain was inhibited in a manner identical to the paro-

Table 1
Adenylate cyclase pmoles/mg protein/10 min in presence of

Tissue	No addition	0.5 mM EGTA	0.5 mM EGTA + 0.5 mM CaCl ₂	10 mM NaF	10 mM NaF 0.5 mM EGTA	10 mM NaF 0.5 mM EGTA 0.5 mM CaCl ₂
Liver	ND	ND	ND	540 ± 44	594 ± 30	ND
Kidney	56 ± 4	48 ± 5	ND	659 ± 6	632 ± 23	ND
Brain	1090 ± 60	520 ± 10	1080 ± 20	5520 ± 130	3570 ± 50	4940 ± 100
Parotid	366 ± 14	209 ± 18	313 ± 12	804 ± 56	403 ± 34	660 ± 98
Parotid + Lubrol	336 ± 14	133 ± 4	312 ± 10	615 ± 29	302 ± 11	535 ± 33

Effect of EGTA and EGTA + CaCl₂ on basal and NaF stimulated activities of adenylate cyclase prepared from various mouse tissues. Values are mean ± S.E. of at least three determinations. ND indicates measurements not made. Because of the low activity in homogenates, adenylate cyclase was assayed in 2000 g pellets of liver and kidney. Parotid adenylate cyclase was solubilized by homogenization in 1% Lubrol PX and enzyme was assayed in the 20 000 g supernatant.

tid enzyme (table 1). Adenylate cyclase from calf cerebral cortex has been shown by Bradham, et al. [13] to be inhibited by EGTA, enzyme activity being restored by the addition of Ca²⁺ or Sr²⁺. Johnson and Sutherland [14] recently showed that solubilized adenylate cyclase from rat cerebellum has a requirement for metal ions other than Mg²⁺.

In both parotid and brain, calcium stimulates adenylate cyclase by a hormone independent mechanism since solubilized enzyme from both tissues is inhibited by EGTA. NaF and calcium are thus the only known agents besides ATP and Mg²⁺, to directly affect the catalytic unit of adenylate cyclase.

It would seem that brain and parotid adenylate cyclases are unique in several respects: 1) Both tissues display unusually high basal enzyme activities; 2) the enzymes are inhibited by EGTA; 3) during differential centrifugation the enzymes sediment at *g* forces in excess of 10 000 *g* × 10 min; 4) both enzymes are refractory to prostaglandin E₁.

An increase in membrane permeability to calcium ion is an early event occurring in many tissues that respond to an increase in the level of cAMP. Consequently it has been postulated that both cAMP and calcium are required for initiation of the physiologic response [15]. This is certainly the case in endocrine and exocrine glands in which Ca²⁺ is required for secretion [16]. Though the calcium concentrations used in this study are in excess of known physiological concentrations, one does not know the local

Ca²⁺ concentration in the vicinity of adenylate cyclase. It is possible that calcium ion may regulate adenylate cyclase activity in vivo as has been suggested for the enzyme from cardiac muscle [17]. In the parotid, amylase and calcium are concentrated in the zymogen granules and are both secreted in response to IPR [9]. After differential centrifugation in 0.25 M sucrose containing 10 mM MgCl₂, amylase and adenylate cyclase sediment in the same fraction (D.J. Franks, unpublished observation). Hence prior to amylase secretion adenylate cyclase could be exposed to high local calcium concentrations which would fall immediately after secretion and 'switch off' the enzyme.

Clearly, further studies are necessary to fully elucidate the role of cAMP and Ca²⁺ in the IPR stimulated parotid.

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