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MULTIPLE FORMS OF BRAIN ADENYLATE CYCLASE: STIMULATION BY Mn^{2+}

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Summary

Mn^{2+}-stimulated adenylate cyclase (ATP pyrophosphate-lyase-(cyclizing), EC 4.6.1.1) activity in detergent solubilized preparations from mouse brain. While NaF-stimulated activity was decreased by both solubilization and storage at 0—4°C, the ability of the enzyme to be stimulated by Mn^{2+} was maintained for up to one week. By including Mn^{2+} in the assay of adenylate cyclase in gel fractions after isoelectric focusing, two distinct peaks of enzyme activity (pI_1 = 5.8, pI_2 = 6.4) were detected, suggesting the existence of more than one type of catalytic subunit in mouse brain cell membranes.

The emergence of the concept that adenylate cyclase (ATP pyrophosphate-lyase(cyclizing), EC 4.6.1.1) has a central role in mediating cellular responses to a variety of stimuli [1] has directed a great deal of interest to the study of this enzyme. However, attempts to analyze the molecular properties of adenylate cyclase are usually hindered by the low levels of activity present in most tissues and the extreme lability of the enzyme. Separation of brain adenylate cyclase by isoelectric focusing on polyacrylamide gels has previously been used to identify a single broad peak of enzyme activity [2]. In the present studies, utilization of Mn^{2+} has resolved at least two distinct peaks of adenylate cyclase activity. This report also demonstrates that Mn^{2+} can be used to optimize and stabilize enzyme activity during the often lengthy procedures required for solubilization, isolation and characterization.

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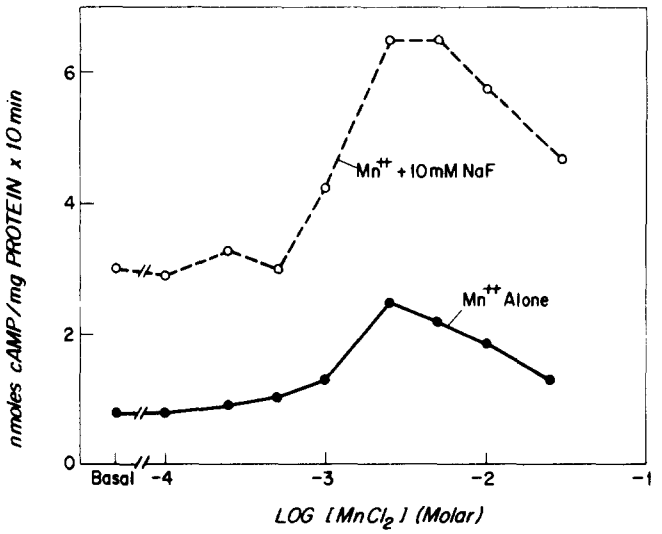

Fig.1. The effect of varying $MnCl_2$ concentration on basal (●—●) or 10 mM NaF-stimulated (○---○) adenylate cyclase activity (nmol/mg protein X 10 min) in brain cell membranes. Whole mouse brains were homogenized in 0.25 M sucrose, 50 mM Tris•HCl, pH 7.5 and centrifuged at 2000 X *g* for 10 min. The supernatant was centrifuged at 200 000 X *g* for 30 min and the pellet (particulate membranes) washed once and suspended in the same buffer. The adenylate cyclase assay medium contained 50 mM Tris•HCl, pH 7.5, 5 mM $MgCl_2$, 2 mM cyclic AMP (cAMP), 1 mM EDTA, 0.0075% bovine serum albumin, 2.5 mM β-mercaptoethanol, 0.5 mM ATP, 0.5 μCi [α - ^{32}P] ATP, an ATP-regenerating system consisting of 5.4 mM phospho*enol*pyruvate and 8 μg pyruvate kinase, and 50—100 μg enzyme protein in a final volume of 0.1 ml. Incubations were carried out for 10 min at 37° C. The reaction was terminated, cyclic [^{3}H] AMP added to determine recovery, and product was isolated on columns (Dowex and Alumina) as described previously [2]. Each point is the mean of triplicate determinations.

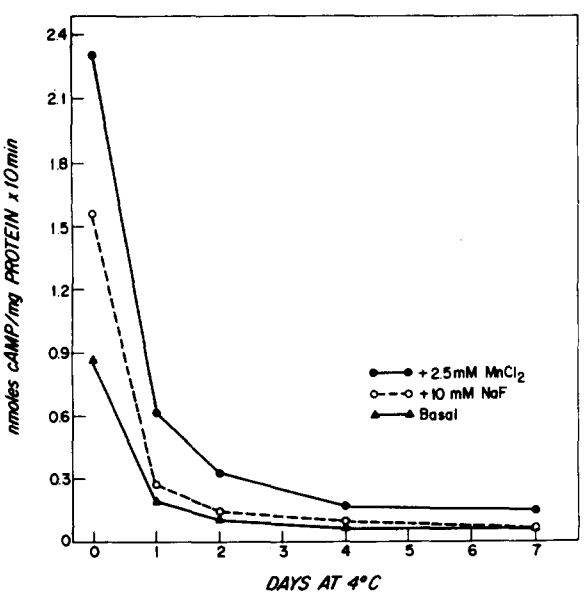

Fig. 2. Effect of storage at 4° C on adenylate cyclase activity in solubilized cell membranes from brain. Particulate membranes prepared as described in Fig. 1 were stirred in 1% Lubrol PX, 0.25M sucrose, 50 mM Tris•HCl for 10 min on ice and centrifuged at 200 000 X *g* for 1 h. This treatment solubilized about 50% of the total protein. Solubilized preparations were stored at 4° C for up to 7 days. At the indicated times, basal, 2.5 mM $MnCl_2$-stimulated, and 10 mM NaF-stimulated adenylate cyclase activity were assayed in triplicate as described for Fig. 1.

As shown in Fig. 1, Mn^{2+} markedly enhanced basal adenylate cyclase activity in cell membrane preparations from mouse brain. The effect was observed over a concentration range of 0.25—25 mM with an optimum at 2.5 mM. Fig. 1 also shows that the effect of 10 mM NaF in combination with Mn^{2+} was approximately additive over the concentration range tested.

In preparations of particulate membranes, 2.5 mM Mn^{2+} stimulated basal activity about 3.5-fold, as did 10 mM NaF. However, the response of the enzyme to F^- was greatly diminished (from a 4-fold to only a 1.25-fold stimulation) by detergent solubilization of membranes, while the response to Mn^{2+} remained the same (approx. 3.5 fold) even after solubilization. Furthermore, the ability of the enzyme to be stimulated by Mn^{2+} was retained during long periods of storage of these solubilized preparations (Fig. 2), while the F^- response was greatly diminished. By storing the solubilized preparations in Mn^{2+} and then conducting subsequent assays of the enzyme in the presence of Mn^{2+} at concentrations optimal for stimulation of activity, it was possible to measure significant adenylate cyclase activity even after seven days at 0—4° C (Fig. 3).

The Mn^{2+} response was also retained after isoelectric focusing of detergent solubilized proteins in polyacrylamide gels. If Mn^{2+} was not included in the assay of adenylate cyclase in the gel fractions, a broad peak of basal activity was observed spanning an isoelectric pH range of 5.2—6.9 [2]. As shown in Fig. 4, the stimulatory effect of Mn^{2+} in these circumstances allowed resolution of the broad peak into multiple adenylate cyclase activities. The total enzyme activity was usually increased about 5-fold revealing at least two distinct peaks of activity. The average p*I* for the first peak was 5.8 with a range of 5.6—5.9 (6 gels from 3 experiments); the average p*I* for the second peak was 6.4 (range 5.9—6.7). Previous investigations into

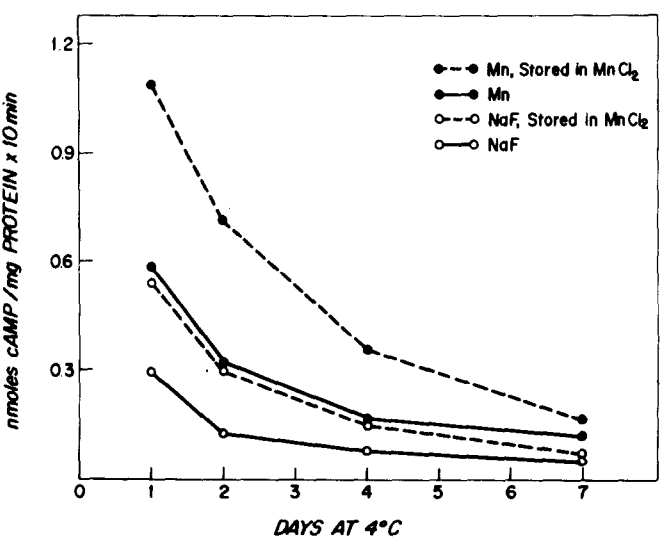

Fig. 3. Effect of storing solubilized membranes in 2.5 mM $MnCl_2$ on adenylate cyclase activity. Solubilized membranes prepared as described in Fig. 2 were stored with or without 2.5 mM $MnCl_2$ for 7 days. At the indicated times, adenylate cyclase activity was measured as described in Fig. 1, but, with a final concentration of 2.5 mM $MnCl_2$ or 10 mM NaF in the reaction mixture.

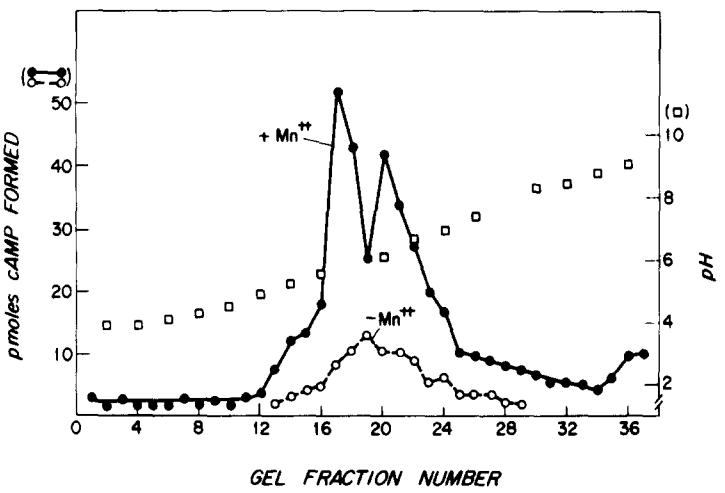

Fig. 4. Effect of 2.5 mM $MnCl_2$ on adenylate cyclase activity assayed after isoelectric focusing. About 500 μg of solubilized membrane protein (prepared as described in Fig. 2) was layered onto a 4% polyacrylamide gel containing 2% ampholines (pH 4—9) and isofocused at 4°C, for 16 h. [2]. 2 mm fractions of duplicate gels were assayed for adenylate cyclase activity, one gel with (●——●) and the other without (○---○) 2.5 mM $MnCl_2$ added to the incubation medium. Other conditions of the assay were identical to those given in Fig. 1, except that the ATP concentration was 0.1 mM and the incubation time was 15 min. A third gel was used for determining the pH gradient.

the molecular properties of solubilized adenylate cyclase have relied on gel filtration and sedimentation characteristics [3—9]. These classic methods have yielded varied estimates of molecular weight for the enzyme from several tissues. In porcine renal cortex [8] and rat renal medulla [9] enzyme activity was found in association with two distinct molecular weight species. Isoelectric focusing provides a unique approach to this general question, since the p*I* of a protein depends on the net charge of the molecule rather than molecular size. Even if molecular weights are similar, subtle structural differences of molecular species may be detected.

We have also examined the effect of Mn^{2+} on adenylate cyclase activity in preparations of cell membranes from mouse parotid gland and liver as compared to brain. Although there were differences in the effective concentration range and in the maximum stimulation elicited by Mn^{2+}, the effect was similarly concentration dependent with an optimum of 2.5 mM in all three tissues.

A detailed analysis of the mechanism by which Mn^{2+} stimulates adenylate cyclase has been considered by others [10, 11] and was beyond the scope of this study. However, from our results, it is apparent that Mn^{2+} acts on the catalytic subunit of adenylate cyclase since it was effective in detergent solubilized preparations and after isoelectric focusing of membrane proteins. It is possible, however, that the moiety separated on polyacrylamide gels contains other components in addition to the catalytic unit of the enzyme. Mn^{2+} probably acts at a site different from that of F^-, since stimulation of the enzyme by these two ions was additive in intact membranes and since the behavior of the enzyme to F^- as compared to Mn^{2+} differed after solubilization and storage.

The use of Mn^{2+} to detect two peaks of adenylate cyclase activity after isoelectric focusing suggests there may be more than one type of catalytic subunit present in brain. This could be a consequence of the mixed cell types in whole brain, or possibly due to mixed populations of adenylate cyclase in one cell type. The existence of multiple catalytic subunits could be hypothesized as contributing to the mechanism(s) whereby cyclic AMP levels might be regulated differently in different cell types, or in the same cell depending on the specific stimulus.

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