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INFLUENCE OF LITHIUM IONS ON EPINEPHRINE-STIMULATED ADENYLATE CYCLASE ACTIVITY IN CULTURED GLIAL TUMOR CELLS

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SUMMARY

1. Adenylate cyclase associated with a particulate fraction of rat glial tumor tumor cells (clone C_6) exhibited optimum activity at Mg^{2+}: ATP ratios near 2. In the presence of excess Mg^{2+}, the enzyme exhibited a K_m for ATP of 0.14 ± 0.04 mM.

2. Li^+ enhanced the effect of epinephrine on adenylate cyclase activity in glial cell particles up to 2-fold but only at Mg^{2+}: ATP ratios greater than 1. Li^+ increased the V of adenylate cyclase but did not alter the apparent K_m for ATP. The influence of Li^+ on epinephrine-stimulated enzyme activity also was observed in intact cells.

3. [Ethylenebis (oxyethylenenitrilo)]tetraacetic acid (EGTA) enhanced adenylate cyclase activity in the presence of various combinations of epinephrine and Li^+ apparently by chelating Ca^{2+}.

4. The influences of Li^+ and Ca^{2+} on adenylate cyclase activity associated with glial cells was different from that reported for brain cortical slices and homogenates, suggesting that other cell types also contribute to adenylate cyclase activity in brain.

INTRODUCTION

In brain slices, the accumulation of cyclic AMP is stimulated by catecholamines of the β-adrenergic type[1,2]. Li^+ inhibits this reaction[3], whereas adenosine and monovalent cations such as K^+ potentiate catecholamine-stimulated accumulation of cyclic AMP[4–6]. K^+ promotes the release of adenosine[4], prompting the suggestion that the influence of K^+ may be mediated by adenosine[6]. Observations by Rall and Sattin[5], however, suggest that at least some of the influence of K^+ may be independent of adenosine. In addition, Ca^{2+} is required for optimum accumulation of cyclic AMP[6]. As suggested from studies with brain homogenates, catecholamines may increase the intracellular concentration of cyclic AMP by stimulating adenylate cyclase activity[7,8].

Abbreviation: EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid.

Li^+ inhibits adenylate cyclase activity in brain homogenates whereas Na^+ and K^+ are without effect[3,9]. In subcellular fractions, both Ca^{2+} and Mg^{2+} are required for optimum enzyme activity[10,11]. Thus catecholamines, monovalent cations, divalent cations, and adenosine may participate in an integrated fashion to influence the intracellular accumulation of cyclic AMP in brain. At least some of these agents appear to influence adenylate cyclase activity directly.

Brain tissue, however, is heterogeneous with glia comprising a large fraction[12]. Cloned lines of astrocytoma cells propagated *in vitro* provide pure populations of glial cells[13,14] and have been used to assess the contribution of glia to measured levels of adenylate cyclase activity in brain. In intact glial tumor cells[15–17], and in glial tumor cell homogenates[18], lysates[19], and particles[20], adenylate cyclase activity is stimulated by β-adrenergic agonists. The specific activity of adenylate cyclase in glial cells is of the same order of magnitude as that found in brain[18,21], suggesting that glia contribute significantly to measured levels of the enzyme activity. In contrast to its inhibitory effect in brain tissues, Li^+ potentiates catecholamine-stimulated enzyme activity in glial tumor cell homogenates[18]. Other monovalent cations in the order $Li^+ > Na^+ > K^+ >$ none also potentiate catecholamine-stimulated adenylate cyclase activity[18]. The data presented here describe additional properties of adenylate cyclase associated with rat glial tumor cells and the influence of Li^+ on these properties.

METHODS

Experiments were conducted with a cloned line of glial cells, designated C_6, derived from a chemically induced rat astrocytoma[13]. Cells were grown in monolayer culture to confluence as described previously[18]. Cultures were screened routinely for aerobic and anaerobic bacteria and for mycoplasma[22] to ensure that they were not contaminated.

Cell homogenates were prepared in 20 mM Tris–HCl (pH 7.7) containing 1 mM $MgCl_2$ and 1 mM dithiothreitol[18]. Particles were prepared by centrifuging homogenates at 11 000 $\times$ g for 10 min, and were resuspended in 0.5 vol. of buffer.

In cell homogenates and in particles, adenylate cyclase activity was determined by measuring the conversion of [2-^{3}H]ATP to cyclic [^{3}H]AMP as described previously[18]. Freshly prepared enzyme (approx. 70 μg of protein in 25 μl) was added to each reaction tube containing an incubation mix of 1 mM ATP, [2-^{3}H]ATP (approx. 10^6 cpm), 6 mM theophylline, 2 mM $MgCl_2$, 50 μg albumin, and 12.5 mM Tris–HCl (pH 7.7) in final volume of 85 μl. Upon completion of the reaction, cyclic AMP was separated from interfering compounds by chromatography on Dowex 50 and by treatment with $BaSO_4$ (ref. 23).

In intact cells, adenylate cyclase activity was measured after labeling endogenous ATP by prior incubation with [8-^{14}C]adenine[24]. Cells for assay were grown as monolayer cultures in 60-mm dishes. F10 growth medium was replaced with 2 ml of Eagle's minimal medium (Grand Island Biologicals) supplemented with 15% horse serum, 2.5% fetal calf serum (2 ml per dish) and 1 μCi of [8-^{14}C]adenine. The dishes were incubated at 37 °C for 1 h. The monolayers were rinsed five times with 2 ml of buffer containing 250 mM sucrose, 5 mM $MgCl_2$, and 20 mM Tris–HCl (pH 7.7); and then incubated with 1.2 ml of the same buffer for 10 min. At the end of the incubation, 0.2 ml of a recovery mix (40 mM ATP + 12 mM cyclic AMP) was added to

each dish. The cells were scraped from the dishes, homogenized at 0 °C, and boiled for 3 min. Insoluble proteins were removed by centrifugation. The amount of cyclic [^{14}C]AMP accumulated was measured in duplicate portions of the supernatants as described previously[23].

MATERIALS

Crystalline disodium ATP, Tris·ATP, cyclic AMP, L-epinephrine, bovine albumin, and Tris were purchased from Sigma; analytical grade Dowex 50 and dithiothreitol from Calbiochem; and [ethylenebis(oxyethylenenitrilo)]tetraacetic acid (EGTA) from Eastman. [2-^{3}H]ATP, 20 Ci/mmole, and [8-^{14}C]adenine, 59 Ci/mole, were purchased from Amersham/Searle. Cyclic [8-^{3}H]AMP, 16.3 Ci/mmole, was purchased from Schwarz Bioresearch. Other chemicals were reagent grade.

RESULTS

Effects of Li^+ and adenosine

Virtually all of the catecholamine-sensitive adenylate cyclase activity was associated with the particulate fraction of glial cell homogenates. In glial cell particles, epinephrine stimulated adenylate cyclase activity 18-fold (Table I). LiCl (10 mM) had a small effect on adenylate cyclase activity measured in the absence

TABLE I

INFLUENCE OF EGTA ON ADENYLATE CYCLASE ACTIVITY IN GLIAL TUMOR CELL PARTICLES

Particles prepared in the presence or absence of EGTA (2 mM) were examined for adenylate cyclase activity in 5-min incubations. The concentration of EGTA in the final reaction mix was 0.6 mM. Epinephrine (0.33 mM), LiCl (10 mM), and $CaCl_2$ (0.6 mM) were added as indicated. Results are expressed as means ± S.E. of 4 experiments.

Additions	*Cyclic AMP accumulated (pmoles/5 min per mg protein)*	
	−EGTA	*+EGTA*
−Epinephrine		
None	11 ± 2	25 ± 3
LiCl	20 ± 1	34 ± 5
$CaCl_2$	17 ± 2	—
+Epinephrine		
None	197 ± 4	314 ± 23
LiCl	360 ± 22	511 ± 29
$CaCl_2$	35 ± 12	—

of epinephrine and potentiated epinephrine-stimulated adenylate cyclase activity (Table I). In the same experiments (data not shown) adenosine (1.0 mM) had no effect on basal or epinephrine-stimulated adenylate cyclase activity. These observations indicate that the influence of monovalent cations, particularly Li^+, on adenylate cyclase activity in glial tumor cells is not mediated by adenosine. Adenosine also had no effect on the accumulation of cyclic AMP in intact glial tumor cells[25].

Effects of divalent cations

Because Li^+ bears some resemblance to Ca^{2+} and Mg^{2+} in chemical reactivity[26], the influences of Ca^{2+} and Mg^{2+} on adenylate cyclase activity were examined. The chelating agent EGTA (2 mM) added to particles prepared from glial tumor cells increased adenylate cyclase activity in the presence of the various combinations of epinephrine and LiCl (Table I). EGTA may stimulate adenylate cyclase activity by chelating Ca^{2+}, since Ca^{2+} potently inhibited epinephrine-stimulated adenylate cyclase activity (Table I). The inhibition by Ca^{2+} was observed in the presence of excess Mg^{2+}, suggesting that Ca^{2+} and Mg^{2+} act separately. In subsequent experiments, EGTA was included in the reaction mixture to achieve optimum enzyme activity.

In the presence of 1 mM ATP, epinephrine-stimulated adenylate cyclase activity in glial cell particles increased with increasing concentrations of Mg^{2+} (Fig. 1). Optimum activity was observed at about 2 mM Mg^{2+}. Li^+ (10 mM) potentiated epinephrine-stimulated enzyme activity up to 2-fold but only at Mg^{2+}

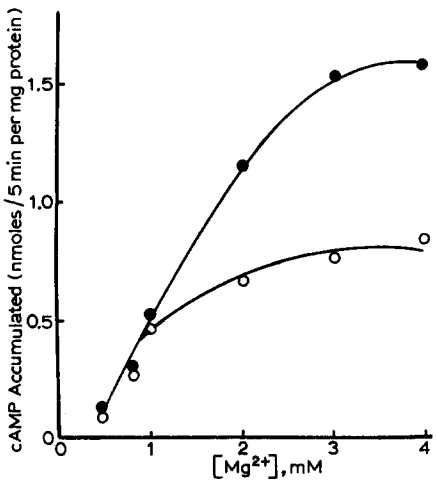

Fig. 1. Influence of Mg^{2+} on adenylate cyclase activity. Adenylate cyclase activity associated with glial cell particles was assayed in a 5-min incubation in the presence of 0.33 mM epinephrine, 1.0 mM ATP, and 0.6 mM EGTA with varying concentrations of $MgCl_2$. Activities were determined in the absence (○—○) or presence (●—●) of 10 mM LiCl. The results of a typical experiment are reported.

concentrations greater than 1 mM (Fig. 1). A similar dependence on Mg^{2+} concentration was observed at 2 mM and 50 mM LiCl. In the presence of 0.5 mM ATP (data not shown) adenylate cyclase activity was optimal at about 1 mM $MgCl_2$, and Li^+ potentiated epinephrine-stimulated enzyme activity only at concentrations of Mg^{2+} greater than 0.5 mM. These observations indicate that adenylate cyclase activity associated with glial particles is optimal at Mg^{2+}:ATP ratios of 2, and that the effect of Li^+ on epinephrine-stimulated adenylate cyclase activity is seen only with Mg^{2+}:ATP ratios greater than 1.

Influence of ATP

In the presence of 3 mM $MgCl_2$, adenylate cyclase activity associated with glial particles increased with increasing concentrations of ATP from 0.04 mM to

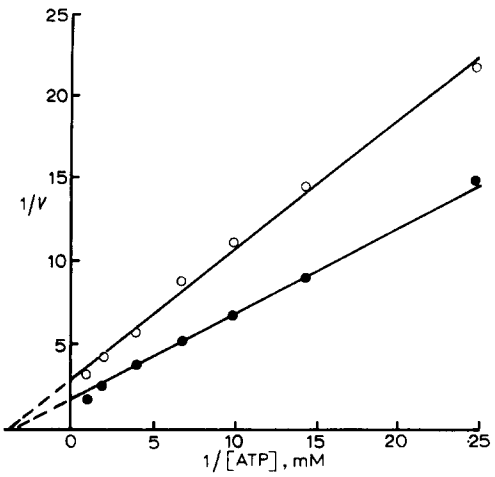

Fig. 2. Influence of ATP on adenylate cyclase activity. Adenylate cyclase activity in glial cell particles was assayed in a 5-min incubation in the presence of 0.33 mM epinephrine, 0.6 mM EGTA, 3 mM $MgCl_2$, and varying concentrations of ATP (added as the Tris salt). Activities were determined in the absence (○—○) or presence (●—●) of 10 mM LiCl. Results of a typical experiment carried out in triplicate are reported and expressed as reciprocals of adenylate cyclase activity (V, nmoles cyclic AMP accumulated/5 min per mg protein) and ATP concentration.

1.0 mM (Fig. 2). As determined from three separate experiments, the enzyme exhibited an apparent K_m for ATP of 0.14 ± 0.04 mM. This value is in agreement with the value of 0.11 mM reported by Jard *et al.*[20]. Li^+ (10 mM) stimulated adenylate cyclase activity at all concentrations of ATP examined. In the presence of Li^+, the enzyme exhibited an apparent K_m for ATP of 0.16 ± 0.06 mM.

Effect of Li^+ on intact glial cells

Inasmuch as some ions, notably F^-, influence adenylate cyclase activity only in broken cells[27], it was of interest to determine if Li^+ could influence adenylate cyclase activity in intact C_6 cells. The experiments with glial cell particles indicated that the influence of Li^+ depended upon Mg^{2+} concentrations in excess of the ATP concentration (Fig. 1). In separate experiments, intracellular concentrations of ATP were estimated at 2.5 mM. In the presence of 5 mM $MgCl_2$, epinephrine increased the intracellular accumulation of cyclic AMP 47-fold (Table II). Li^+ enhanced the

TABLE II

INFLUENCE OF EPINEPHRINE AND Li^+ ON THE ACCUMULATION OF CYCLIC AMP BY INTACT CELLS

The accumulation of cyclic AMP in intact cells was measured in the presence or absence of epinephrine (440 μM) and LiCl (14 mM). The results were normalized to a value of 1.0 for unstimulated levels of adenylate cyclase activity and were expressed as mean relative activities ±S.E. in 3 experiments. In unstimulated samples, 520 ± 88 cpm were accumulated per mg protein in a 10-min incubation.

Additions	*Relative activity*	
	−LiCl	*+LiCl*
None	1.0	0.9 ± 0.2
Epinephrine	47.2 ± 3.3	73.3 ± 0.3

effect of epinephrine on the accumulation of cyclic AMP in intact glia with little effect on unstimulated enzyme activity (Table II).

DISCUSSION

The observations reported here suggest that adenylate cyclase from glial tumor cells, like the enzyme from brain, is influenced by a complex interaction of catecholamines, ATP, and monovalent and divalent cations. Under the conditions of the experiments described herein, ATP and Mg^{2+} likely formed an equimolar complex[28], and participated in the complexed form as substrate for adenylate cyclase[29–31]. The requirement for Mg^{2+} at concentrations in excess of the ATP concentration for optimal adenylate cyclase activity (Fig. 1) has been interpreted to reflect an association of Mg^{2+} with alternate sites as well[29,30]. The requirement for Mg^{2+} concentrations in excess of the ATP concentration for the potentiating effects of Li^+ (Fig. 1) suggests that the action of Li^+ may depend upon the action of Mg^{2+} at the catalytic site and at a minimum of one additional site. The apparent effect of Li^+ was to increase the V of adenylate cyclase without altering the K_m for ATP (Fig. 2). Based on observations that Li^+ potentiated catecholamine-stimulated adenylate cyclase activity but influenced fluoride-stimulated enzyme activity only marginally, we previously suggested that Li^+ may influence the action of the catecholamine receptor on the catalytic subunit of the adenylate cyclase system[18]; however, inasmuch as Li^+ increased the relative activities of both unstimulated and epinephrine-treated samples 1.8-fold (Table I) we cannot exclude the possibility that Li^+ affects the expression of the entire enzyme system. Interpretations of these kinetic data, however, have to be viewed cautiously, because of the limitations imposed by the crude, particulate nature of the enzyme preparation.

The influences of Li^+ and Ca^{2+} on adenylate cyclase from glial tumor cells were markedly different from their effects on the brain enzyme[3,6,10,11]. These observations suggest that adenylate cyclase from glial tumor cells is not completely representative of the enzyme from brain tissue. The differences could reflect altered properties of glial tumor cells maintained *in vitro*. On the other hand, since C_6 cells have retained at least some differentiated characteristics[13,15,18–20,32,33], the effect of Li^+ and Ca^{2+} on adenylate cyclase activity in C_6 cells as reported here may be representative of influences of these cations on glia *in situ*. The differences in behavior of adenylate cyclases from glial and cerebral cortical tissue may reflect the contributions of other cell types in brain to measured levels of enzyme activity[34,35].

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