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Effect of Lithium on Phosphodiesterase in Rat Brain

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Abstract

The invitro effect of lithium on phosphodiesterase (PDE) was studied in rat brain. There was a concentration dependent inhibition of basal level of both high Km and low Km PDE by lithium chloride. There was a significant (10-30%) inhibition of basal PDE (high Km) with 3 mM LiCl in various regions of rat brain. The effect was more pronounced in medulla (32%), mid brain (24%) and pons (21%). It is observed that at therapeutically relevant levels there is no significant effect of lithium on cerebral PDE. However, at concentrations above 3 mM, lithium shows a significant inhibition of PDE and the effect is pronounced in certain regions of rat brain.

Key words -
Phosphodiesterase,
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Since Cade [1] first introduced lithium salts in the treatment of mania several workers have made extensive studies on the mechanism of action [2], [3], therapeutic utility [4], [5] and prophylactic use [6] of lithium in mania. Inspite of various studies on the pharmacokinetics of lithium [7], the mechanism of action of lithium in brain has not been studied so extensitvely. To explain the basic biochemical mechanism involved for the mode of therapeutic action of lithium a regulatory molecular loop lesion correction hypothesis has been suggested. Considering the complementarity of the immune, nervous and endocrine systems, dysregulation in any one of these will affect alterations in other [8]. Molecules like calcium, cyclic nucleotides, peptides and other second messengers could contribute to the functional integrity of the loop. The reversal of the dysregulation of these three systems has been suggested as one of the biochemical mechanisms involved in the action of lithium.

A number of investigations have now focused on the effect of long term treatment with lithium on a variety of

biochemical events in the central nervous system. Evidences are now accumulated to suggest that lithium action might be through inhibition of signal transduction mediated by phosphoinositide [9]; through transcriptional regulation via activation of protein kinase C and/or cAMP dependent kinases [10], or through G-protein-coupled second messenger system [11]. The basal expression on neuropeptide -Y (NPY), which has been reported to be directly involved in the pathogenesis of mood disorder [12], has also been shown to be enhanced by lithium in rat hypothalamus. This NPY is regulated by second messenger system and is induced by agonist that stimulate cAMP [13]. Though it is well established that certain divalent cations act on enzymes involved in cAMP homeostasis like adenyl cyclase (AC) and phosphodiesterase (PDE), the action of monovalent cations like lithium on these enzymes has not been fully worked out. In view of these reports and the fact that second messenger system, especially cAMP, might be involved in mood correcting affect of lithium in mania, experiments were conducted to study the invitro effect of lithium on cAMP metabolizing enzyme phosphodiesterase in rat brain.

Materials and Methods

Adult male Sprague Dawley rats, weighing approximately 200-250 gm, were used in the study. The animals were allowed free access to rat chew and water. Animals were sacrificed by decapitation and brains removed. Regions were dissected on ice adopting the procedure described by Glowinsky and Iverson [14]. Tissues were homogenized in ice cold 50 mM tris-HCI buffer (pH 8.0) and the homogenate was used for phosphodiesterase assay as per the procedure described by Weiss [15]. An aliquote equal to 0.5 mg protein was incubated in reaction mixture (1.0 ml) containing, in final concentration, 50 mM tris-buffer (8.0 pH); 0.5 X 10^{-3} M cAMP; 3 mM MgCl2 and alkaline phosphatase (4 units), at 37° C for 30 minutes. The reaction was terminated by 55% TCA and liberated Pi in the supernatant was measured by single reagent PAMD [16] at 318 nm in a spectrophotometer. PDE was assayed in presence of various concentrations of lithium. PDE was also assayed in presence of different concentrations of NaCl and KCl, with and without $MgCl^2$ in the assay system.

Low Km PDE was assayed at different concentrations of LiCl using partially purified enzymes source. The enzyme was partially purified from rat brain. In brief: brain tissues obtained from adult rats were homogenized (1:25 w/v) in cold 20 mM, tris-HCI buffer containing 1 mM EDTA, pH 7.5. The homogenate was centrifuged and proteins precipitated for half saturation with ammonium sulfate. The resultant precipitate dissolved in tris buffer (pH 7.5) was dialyzed and applied on DEAE-Cellulose column. PDE was eluted with a linear salt gradient with 0.05 and 0.4 M NaCl and the elute was monitored at 280 nm in a UV detector attached to the fraction collector. Fractions with highest absorbance at 280 nm were pooled and dialyzed. This elute was rechromatographed on DEAE-Cellulose column and eluted similarly. Elutes at 0.11 and 0.21 M NaCl were pooled and further purified by desalting using Biogel-P-6-DG column and eluted with 0.11 M NaCl. Fractions with highest UV absorbance at 280 nm were pooled and lyophylized. The lyophylized PDE was reconstituted in assay buffer and used in the assay. Protein is determined by Lowry's [17] method and the activity was expressed as micromoles of Pi liberated per mg protein per 30 minutes.

Results

Phosphodiesterase, both high Km (crude homogenate) and low Km (partially purified), activity was assayed in whole brain as well as in various regions of rat brain. When high Km PDE was assayed in

presence of lithium alone it was observed that 3 mM LiCl showed maximal activity in whole brain though the activity was nearly 10% less than with 3 mM MgCl2 (basal). However low Km PDE showed maximal activity at 2 mM LiCl when compared to maximal activity with 3 mM MgCl2. There is a concentration dependent inhibition in basal activity of both high Km and low Km PDE with increasing lithium concentration. Maximum of 12% inhibition is seen with 5 mM LiCl (Figure 1). No significant effect on PDE was observed with either NaCl or Kcl at equivalent concentrations of LiCl (Table 1).

Effect of lithium on phosphodiesterase in rat brain. Enzyme activity was assayed in crude homogenate (left) as well as with partially purified enzyme source (right), as described in methods, in presence of 0.5 × 10^{-3} M (high Km) and 0.5 × 10^{-6} M (low Km) cAMP; with various concentrations of LiCl with (Δ - Δ) and without (● - ●) 3 mM MgCl2. (Mean values obtained from six experiments, each in duplicate are plotted)

Table I - Effect off NaCl and KCl on PDE

PDE was assayed as described with 0.5 × 10-3M cAMP and various concentrations of NaCl or KCl in presence (B) or absence (A) of 3.0 mM MgCl2

×in rat brain

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High KM PDE activity was also assayed in different regions of rat brain in presence of either 3 mM MgCl2 (basal) or 3 mM LiCl. It is observed that striatum has the highest basal PDE activity, whereas cerebellum showed lowest activity (50% of striatum). When compared to striatum, thalamus and hippocampus showed 92%, cortex and pons, 80% and midbrain and medulla showed 70% of basal activity. Nearly 5-18% lower activity of PDE was observed in different regions of rat brain with 3 mM LiCl alone when compared to basal activity (Table 2). However, a significant inhibition (10-30%) of basal PDE was seen with 3 mM LiCl in various regions of brain. Medulla showed maximum (32%) inhibition with 3 mM LiCl.

Table II - Effect of lithium on regional distribution of PDE in rat brain

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Unit: micromoles of cAMP hydrolysed/mg protein/30 minutes.

Values are mean ± SD of three estimations, each in duplicate, of each region pooled from three rats. PDE was assayed with 0.5 × 10^{-3} cAMP and 3 mM LiCl in presence (C) or absence (B) of 3 mM MgCl2. Basal PDE was assayed in presence of 3 mM MgCl2 (A).

(a) When compared to basal and with LiCl only

(b) When compared to basal and with MgCl2 and LiCl.

Discussion

Lithium has been extensively and effectively used as prophylactic measure in manic depressive psychosis. However, the mechanism of action of lithium is poorly understood. Apart from various mechanisms that have been postulated for its action on peripheral and central nervous system [6], [7], evidences are now accumulating to suggest that lithium action might be mediated through cAMP dependent kinases [10] ad G-protein coupled cAMP system [11]. It is evident from these reports that the action of lithium might also depend on the homeostatis of cAMP in brain. Though lithium has been shown to inhibit the rise in cAMP levels in brain via the inhibition of adenylcyclase [18], no reports are available in literature on the effect of lithium on enzymes metabolizing cAMP. It is suggested that neuroleptics exert their effect via the inhibition of PDE and since lithium and neuroleptics have one therapeutic effect - antimanic action - in common, it may be relevant to study the effect of lithium on PDE.

The effect of lithium on both high Km and low Km PDE has been studied in rat brain. Though in brain the cAMP hydrolysis by low Km PDE is very low, certain antipsychotic drugs and divalent cations have been shown to be more effective on low Km PDE than on high Km PDE. Though it is observed that high Km PDE is not significantly affected by lithium, it is found to be equally effective in expressing the enzyme activity when compared to Mg ions. However, the basal activity of both high Km and low Km PDE is inhibited by increasing the lithium concentration. Lithium has been shown to interfere with magnesium sensitive sites on adenylcyclase [19]. The inhibition of PDE seen in this study might also be due to its interaction with magnesium sites. Nearly 30% of low Km enzyme activity is inhibited by 5 mM LiCl concentration. This concentration in humans is however in toxic range. In the therapeutic range of 0.5-2 mM concentration there is no significant inhibition of high Km PDE in rat brain. However, low Km PDE is inhibited even at this concentration of lithium. It could be stipulated that at higher concentrations of lithium the cAMP hydrolysis is inhibited via high Km PDE thus increasing the accumulation of cAMP in brain. It is observed that neither NaCl nor KCl at equivalent concentration of LiCl used, has any effect on basal PDE. Naharski et al [20] have shown that at therapeutic levels of lithium, brain phosphoinositol is increased via inhibition of inositol monophosphatase in brain.Similarly adenylcyclase has also been shown to be inhibited by lithium at lower concentrations [18]. Our results show that at therpeutically relevant levels of lithium i.e 0.5-2.0 mM, cAMP hydrolysis is not affected. Since the cAMP hydrolysis by low Km PDE is very small in brain the significant inhibition of low Km PDE by lithium seen in this study would not result in significant rise in cAMP levels in brain. However, at higher concentrations of lithium cAMP accumulation would increase via the inhibition of both low Km and high Km PDE.

The regional effect of lithium on high Km PDE in rat brain is variable. In presence of 3 mM lithium the expression of PDE itself is 10-15% less than basal PDE. Nearly 10-30% inhibition of basal PDE is observed in various regions with 3 mM lithium. The effect of lithium on basal PDE is more pronounced in medulla (32%), midbrain (24%) and pons (21%). It is speculated that at higher concentrations of lithium (>3 mM) not only the cAMP hydrolysis (by both high Km and low Km PDE) is inhibited in whole brain, but also the effect is more pronounced in certain regions of brain. It seems probable that lithium at therapeutic levels exerts its effects invivo via modulation of the phosphoinositol cycle, as reported [20], but at higher levels lithium affects cAMP system. Higher concentration of lithium (> 3 mM) results in accumulation of cAMP in certain regions of rat brain. It is essential to know whether these effects are also seen in invivo conditions. Experiments are underway involving treatment of animals with lithium salts. It is evident from this invitro study that at therapeutically relevant levels,

lithium action is not via cAMP second messenger system. However, at higher doses this system is affected via the inhibition of cerebral PDE and the effect is regional. However, the question whether the effect of lithium on PDE is of relevance for its antimanic properties remains to be answered.

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