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EFFECTS OF LITHIUM ON BRAIN ADENYL CYCLASE ACTIVITY

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Abstract—Sodium fluoride-induced adenyl cyclase activity of rat and rabbit cerebral cortex homogenates is inhibited by a wide range of concentrations of lithium. Lithium also inhibits NE-induced formation of cyclic AMP in cerebral cortex slices of rat, and histamine-induced formation of cyclic AMP in cerebral cortex slices of rabbit. The effects of lithium are already evident at a 2 mM concentration, and are specific for this ion. This concentration is easily achieved during the treatment of acute manic patients with lithium, suggesting that the psychosedative effects of lithium may be related to the inhibition of brain adenyl cyclase.

FAVORABLE reports on the use of lithium salts in the treatment of manic states have appeared.[1–3] Although the administration of lithium to rats does not modify the tissue levels of norepinephrine (NE), dopamine or serotonin,[4] modifications of the turnover rate of NE and serotonin have been observed with chronic lithium administration.[5–7] It has been found also that lithium alters the metabolism of intracisternally injected NE,[8] and that it diminishes the electrically-induced release of NE and serotonin from brain slices.[9] At the present time, it has not yet been established whether these changes in monoamines turnover, metabolism and release are related to the therapeutic effect of lithium in mental disorders.

Recently two different groups have reported an increased urinary excretion of adenosine 3′,5′-cyclic monophosphate (cyclic AMP) in manic patients.[10–12] In a preliminary report it has been stated also that manic-depressive patients who improved on lithium therapy showed significant changes in urinary excretion of cyclic AMP.[13] As we do not know the sources of urinary cyclic AMP, it is impossible to decide whether these changes in cyclic AMP excretion are reflections of changes in the metabolism of the cyclic nucleotide in brain. It is not known either if changes in the brain levels or turnover of cyclic AMP are related to mental illness, and there is no experimental evidence for the conclusion that mania may result from the cellular accumulation of cyclic AMP in brain.[11,13]

Adenyl cyclase, the enzyme that catalyzes the formation of cyclic AMP from ATP, is present in high concentrations in brain. It has been impossible to demonstrate a significant activation of adenyl cyclase by neurohormones in brain homogenates. However, in brain slices, several authors have described an increased formation of cyclic AMP induced by various monoamines.[14–16] Histamine is the most potent inducer of cyclic AMP formation in cerebral cortex slices of rabbit and guinea pig, while in the rat, mouse and cat histamine is inactive and NE is a potent inducer.[17]

Some divalent cations influence the hormone induced activation of adenyl cyclase in several tissues.[18] We report in this paper that the monovalent cation lithium, in very low concentrations, antagonizes the NE and histamine induced formation of

cyclic AMP in brain cortex slices. In brain homogenates, the sodium fluoride stimulated adenyl cyclase is also inhibited by lithium, although higher concentrations are required to obtain the same degree of inhibition as in brain slices.

MATERIALS AND METHODS

Materials were obtained from the following sources: ATP-^{3}H (G) (15·7 c/m-mole), New England Nuclear Corp.; 8-^{14}C adenine (50 c/m-mole), Schwarz BioResearch; adenosine 3′,5′-cyclic monophosphate, Boehringer–Mannheim Corporation; histamine dihydrochloride, choline chloride and lithium chloride, Carlo Erba; *l*-norepinephrine bitartrate, Winthrop Laboratories.

Male New Zealand rabbits (2·0–2·5 kg) and Sprague–Dawley male rats (200–240 g) were killed by decapitation and the brains removed in less than 3 min. Cerebral cortex was homogenized gently by hand in an all glass homogenizer in tris–HCl buffer, pH 7·4. The incubation medium for the assay of adenyl cyclase contained: tris–HCl buffer, pH 7·4, 4×10^{-2}M; $MgSO_4$, $3{\cdot}3 \times 10^{-3}$M; theophylline, 10^{-2}M, NaF, 10^{-2}M; [^{3}H]ATP, 2×10^{-3}M, 20 c/m-mole and 10 mg of tissue in a final volume of 1 ml. When indicated, lithium chloride was added before the [^{3}H]ATP in a small volume to give the final desired concentration. Incubations were carried out at 30° for 10 min and were terminated after the addition of 0·1 ml (0·5 mg) of a solution of carrier cyclic AMP, by immersion in a boiling water bath for 2 min. The [^{3}H]cyclic AMP formed was isolated by ion-exchange chromatography and Ba–Zn precipitation.[19]

For the experiments with cerebral cortex slices, blocks of tissue not thicker than 3–4 mm were rapidly cut after decapitating the animals and were immersed in Krebs–Ringer bicarbonate buffer, pH 7·4, which was gassed with 95% O_2–5% CO_2 and consisted of 119 mM NaCl; 4·8 mM KCl; 25 mM $NaHCO_3$; 1·2 mM KH_2PO_4; 2·6 mM $CaCl_2$; 2·4 mM $MgSO_4$ and 0·1% glucose. Theophylline was not included because we have seen that it does not enhance cyclic AMP accumulation in brain slices.[14,15] Slices 0·25 mm thick were obtained with a McIlwain tissue chopper, and incubated for 30 min at 37° in Krebs–Ringer bicarbonate buffer containing 1 mc of [^{14}C]adenine per ml (2×10^{-5}M). After this preincubation the slices were washed with Krebs–Ringer bicarbonate buffer to remove all excess labelled adenine and distributed into test tubes (12 × 50 mm) containing 0·8 ml of buffer (40–50 mg tissue or 8–10 slices per tube). Before addition of the drugs, the tubes were incubated for an additional time of 30 min at 37° in an atmosphere of 95% O_2–5% CO_2. The drugs, dissolved in buffer, were added in 0·1 ml volumes to give the final desired concentration. When lithium chloride or choline chloride were added, the concentration of sodium chloride was reduced in order to maintain the same total molarity in the medium. The tubes were incubated for 10 min after adding the drugs. The incubation was terminated by addition of 0·4 ml aliquots of a solution containing 0·1 mg cyclic AMP and 0·6 m-moles $HClO_4$. The supernatant fluid was chromatographed on Dowex 50 hydrogen form and the fraction containing the cyclic AMP was subjected twice to $Ba(OH)_2$–$ZnSO_4$ precipitation to remove contaminating nucleotides. The specificity of this procedure to isolate [^{14}C]cyclic AMP from brain slices preincubated with [^{14}C]adenine has been proved in previous publications.[17,20]

The protein content of the slices contained in each tube was measured according to Lowry *et al.*[21] with crystalline bovin albumin as standard.

RESULTS

Effect of different concentrations of lithium on sodium fluoride-activated adenyl cyclase in cerebral cortex homogenates of rat and rabbit. The sodium fluoride-induced formation of cyclic AMP in rat and rabbit cerebral cortex homogenates was linear for more than 20 min. The presence of lithium, at a concentration 2 mM, produced a small decrease in the sodium fluoride-stimulated adenyl cyclase activity of rat and rabbit cerebral cortex homogenates. Higher concentrations of lithium produced more marked inhibition: at 100 mM 71 per cent inhibition in the rat and 77 per cent inhibition in the rabbit was obtained. Similar concentrations of sodium or potassium chloride had no significant inhibitory effects (Tables 1 and 2).

TABLE 1. EFFECT OF LITHIUM ON NaF-ACTIVATED ADENYL CYCLASE OF RAT CEREBRAL CORTEX HOMOGENATES*

Addition	nmoles cyclic AMP /mg tissue/10 min	Inhibition (%)	P value
None	0·505 ± 0·020		
2 mM LiCl	0·474 ± 0·014	6	<0·05
5 mM LiCl	0·422 ± 0·012	17	<0·01
10 mM LiCl	0·377 ± 0·019	25	<0·001
25 mM LiCl	0·277 ± 0·015	45	<0·001
50 mM LiCl	0·209 ± 0·009	59	<0·001
100 mM LiCl	0·147 ± 0·011	71	<0·001
50 mM KCl	0·483 ± 0·016	4	N.S.
100 mM KCl	0·463 ± 0·028	9	<0·05
50 mM NaCl	0·481 ± 0·010	4	N.S.
100 mM NaCl	0·446 ± 0·010	12	<0·05

* The assay of adenyl cyclase was carried out in presence of NaF 10^{-2}M as indicated in the text.

Each value is the average ±S.E. of four experiments. P values compared to group with no addition were established by Student's *t*-test.

N.S., not significant.

Effect of lithium on NE-induced formation of cyclic AMP in cerebral cortex slices of rat. The formation of cyclic AMP in brain slices in response to different neurohormones varies greatly depending on the area of the brain as well as the animal species. The cerebral cortex of the rat is particularly sensitive to NE, and it is completely insensitive to histamine. At 10^{-4}M, NE produces a maximal activation of cyclic AMP formation.[16,17,20] At a 2-mM concentration of lithium in the medium, the NE-induced formation of cyclic AMP is inhibited by 22 per cent, and it is inhibited by 80 per cent at a 50 mM concentration of lithium (Table 3).

Since, in order to maintain in all experiments the same osmolarity in the incubation medium of the slices, the concentration of sodium chloride was decreased to balance the increase in molarity due to lithium chloride, we had to determine also the influence of low concentrations of sodium on the NE-induced formation of cyclic AMP. In these experiments sodium chloride was substituted by equimolar concentrations

TABLE 2. EFFECT OF LITHIUM ON NaF-ACTIVATED ADENYL CYCLASE OF RABBIT CEREBRAL CORTEX HOMOGENATES*

Addition	nmoles cyclic AMP /mg tissue/10 min	Inhibition (%)	P value
None	0·602 ± 0·012		
2 mM LiCl	0·528 ± 0·015	12	<0·01
5 mM LiCl	0·460 ± 0·018	24	<0·01
10 mM LiCl	0·432 ± 0·014	28	<0·001
25 mM LiCl	0·303 ± 0·015	50	<0·001
50 mM LiCl	0·204 ± 0·007	66	<0·001
100 mM LiCl	0·140 ± 0·031	77	<0·001
50 mM KCl	0·614 ± 0·026	0	N.S.
100 mM KCl	0·581 ± 0·028	4	N.S.
50 mM NaCl	0·609 ± 0·034	0	N.S.
100 mM NaCl	0·576 ± 0·030	4	N.S.

* The assay of adenyl cyclase was carried out in presence of NaF 10^{-2}M as indicated in the text.

Each value is the average ±S.E. of four experiments. P values compared to group with no addition were established by Student's *t*-test.

N.S., not significant.

TABLE 3. EFFECT OF LITHIUM ON NE-INDUCED FORMATION OF CYCLIC AMP IN RAT CEREBRAL CORTEX SLICES*

Addition	Counts/min /mg protein/10 min	Inhibition (%)	P value
None	238 ± 21		
NE	2031 ± 88		
NE 1 mM LiCl	1913 ± 103	6	<0·05
NE 2 mM LiCl	1487 ± 84	27	<0·01
NE 5 mM LiCl	1202 ± 56	41	<0·001
NE 10 mM LiCl	1021 ± 47	50	<0·001
NE 25 mM LiCl	559 ± 39	73	<0·001
NE 50 mM LiCl	416 ± 29	80	<0·001
NE 25 mM choline	2040 ± 120	0	N.S.
NE 50 mM choline	1980 ± 134	3	N.S.
50 mM choline	204 ± 26		

* Slices of cerebral cortex were incubated in Krebs–Ringer bicarbonate buffer with 1 mc of [^{14}C]adenine (2×10^{-5}M) for 30 min. The slices were then washed and incubated with or without NE (10^{-4}M) for 10 min in presence of various concentrations of lithium chloride or choline chloride. When lithium chloride or choline chloride were added the molarity of the incubation medium was maintained by the appropriate reduction of the concentration of sodium chloride.

Values for [^{14}C]cyclic AMP formed are the average S.E. of at least eight experiments. P values compared to group with NE alone were established by Student's *t*-test.

N.S., not significant.

of choline chloride. As it is shown in Table 3, the NE-induced formation of cyclic AMP is not significantly modified by the presence of choline chloride 25 and 50 mM. Moreover, choline chloride alone does not modify either the basal formation of cyclic AMP. These data suggest that the inhibition produced by lithium is a specific property of this ion, and that it is not secondary to the decrease in sodium concentration.

Effect of lithium on histamine-induced formation of cyclic AMP in cerebral cortex slices of rabbit. The effects of various concentrations of lithium on the histamine-induced formation of cyclic AMP in cerebral cortex slices of rabbit is shown in Table 4. The concentration of histamine used in these experiments induces a maximal

TABLE 4. EFFECT OF LITHIUM ON HISTAMINE-INDUCED FORMATION OF CYCLIC AMP IN RABBIT CEREBRAL CORTEX SLICES*

Addition	Counts/min /mg protein/10 min	Inhibition (%)	P value
None	54 ± 8		
Histamine	3540 ± 220		
Hist. 2 mM LiCl	2884 ± 185	19	<0·01
Hist. 5 mM LiCl	1972 ± 141	44	<0·001
Hist. 10 mM LiCl	1540 ± 92	57	<0·001
Hist. 25 mM LiCl	1251 ± 107	65	<0·001
Hist. 50 mM LiCl	942 ± 64	73	<0·001
Hist. 50 mM choline	3725 ± 255	0	N.S.
50 mM choline	50 ± 7		

* For experimental details, see Table 3. Histamine concentration was always 10^{-4}M.

Values for [^{14}C]cyclic AMP formed are the average ±S.E. of at least six experiments. P values compared to group with histamine alone were established by Student's *t*-test.

N.S., not significant.

formation of cyclic AMP in cerebral cortex slices of rabbit.[16] A concentration of lithium as low as 2 mM inhibits significantly the histamine-induced formation of cyclic AMP. The extent of the inhibition is progressively greater with increasing concentrations of lithium (Table 4). In presence of choline chloride 50 mM the histamine-induced formation of cyclic AMP is not modified. Also, the basal production of cyclic AMP is not modified by 50 mM choline chloride, showing that a decrease in sodium concentration does not influence the formation of cyclic AMP.

DISCUSSION

The sodium fluoride and hormone-stimulated activity of adenyl cyclase in several tissues is influenced by various cations. It is known that the enzyme requires a divalent cation, like magnesium or manganese, for optimal activity.[18,22] In rat fat cells, the complete removal of calcium blocks the stimulatory effect of ACTH on adenyl

cyclase activity and on lipolysis, while it does not inhibit the effects of glucagon, epinephrine and fluoride.[23,24] Monovalent cations have less influence than divalent cations on adenyl cyclase activity. In fat cells preparations, sodium, potassium and rubidium have little or no effect on fluoride and ACTH-stimulated activity of adenyl cyclase. Lithium, at 100 mM, inhibited fluoride and ACTH-stimulated activities 35 and 52 per cent respectively.[18] The effect of divalent cations on fluoride-stimulated adenyl cyclase from brain homogenates has been well studied, and the results are similar to those obtained in other tissues.[25] But the fact that fluoride usually does not activate adenyl cyclase in intact cells makes the results obtained with fluoride activation in homogenates of doubtful physiological meaning.

Adenyl cyclase of brain homogenates is not significantly stimulated by any hormone. The hormone-induced activity of brain adenyl cyclase has been shown only in brain slices. The first studies made in rabbit and guinea pig demonstrated that histamine is the most potent inducer of brain cyclic AMP formation in these animal species.[15] Studying other animal species, one of us observed that in the rat NE is the most potent activator of adenyl cyclase in brain slices, while histamine is completely inactive.[17,20] Up to date, no reports had been published on the effects of lithium on this system. The development of a simple radioisotopic method to measure the formation of [^{14}C]cyclic AMP from ATP prelabelled with [^{14}C]adenine in brain slices,[16,20] and the current interest on the psychotropic effects of lithium prompted us to undertake a study on the effect of lithium on sodium fluoride-stimulated adenyl cyclase in brain homogenates and on hormone-stimulated adenyl cyclase in brain slices.

We have found that lithium has an inhibitory effect on fluoride-stimulated adenyl cyclase activity in brain homogenates of rat and rabbit, as well as on NE-induced formation of cyclic AMP in rat brain cortex slices and on histamine induced formation of cyclic AMP in rabbit cerebral cortex slices. Since lithium is a highly toxic ion for animals and man, we had to find out the lowest concentration that would still influence brain adenyl cyclase, to make sure that this inhibition could be related to the mechanism of action of lithium in mental illness. We have seen that in homogenates and in brain slices of rats and rabbits a concentration of lithium as low as 2 mM inhibits significantly the induced formation of cyclic AMP by fluoride, NE and histamine. It is important to remark that these concentrations of lithium can be easily reached during the treatment of acute mania with lithium salts.[26]

It has been found by other authors that low concentrations of lithium diminish the release of NE and serotonin from brain slices,[9] and that lithium produces a shift of NE metabolism from *O*-methylation to oxidative-deamination,[8] suggesting that under lithium therapy less NE is released in active form. Current theories based on the relationship between the effects of drugs on NE and affective or behavioral disorders predict that any treatment improving manic symptoms acts by reducing the function of central adrenergic neurons.[27] The diminished release of NE and the inhibition of brain adenyl cyclase could both contribute to produce a central adrenergic blockade that benefits manic and hypomanic disorders. However, from our experiments in animals, the effect of lithium does not seem to be specific for any adenyl cyclase activator, since fluoride, NE and histamine effects are all blocked by lithium, and further investigations are necessary to elucidate if one or more neurotransmitters are involved in the psychotropic effect of lithium.

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