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# Action of lithium on ATPases in the rat iris and visual cortex

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The diverse effects of lithium on adenosine triphosphatase (ATPase, EC 3.6.1.3) activities in different tissues have been discussed by Birch [1]. To obtain these effects in a reproducible manner, it appears to be necessary to administer lithium salts *in vivo*, incubation of various preparations *in vitro* showing either no effect, or an indeterminate one [2]. Previously in this laboratory we have examined the effects of chronic lithium administration on membrane-bound $Na^+/K^+$ activated $Mg^{2+}$-dependent, $Ca^{2+}/Mg^{2+}$ activated and $Mg^{2+}$ activated ATPases in rat cortical synaptosomes [2], human erythrocytes [3], human blood platelets [4] and choroid plexus [5]. We decided to extend our investigations to iris tissue in an attempt to use a relatively simple peripheral tissue, autonomically inervated adrenergically and cholinergically but containing no nerve cell bodies, the nerve supply arising mainly from cell bodies in the superior cervical ganglion and partly from the ciliary ganglion [6] and to compare it with tissue from a well defined central cortical structure containing a large population of neurones, viz. the visual cortex. It was thought that in terms of activity, these two structures might be related. In the literature, data on ATPase activity in the iris is lacking, studies being confined to the ciliary body [7].

*Methods.* All reagents were of Analar grade unless otherwise stated. Disodium ATP was obtained from Boehringer Ltd. and Lubrol was type PX obtained from Sigma Chemical Co. Ltd. Male Wistar rats, kept under conditions of 12 hr light–12 hr dark (not reversed daylight) were given lithium chloride (BDH) in the diet, 60 mM LiCl/kg food for periods up to 4 weeks [2]. The food was standard rat diet, Oxford modified 45B, ground. Control animals received the diet without added lithium salt, but both control and medicated animals were given a free choice of 0.9% sodium chloride solution or water to drink; this procedure keeps the animals healthy [8]. Animals were killed by decapitation, the eyes and brain removed as rapidly as possible, dissected to obtain irides without ciliary body tissue [9] and visual cortices [10]. Ten per cent homogenates of the tissue were made in 0.3 M sucrose containing 1 mM magnesium by homogenising for 90 sec using a glass pestle clearance 0.25–0.38 mm at 1000 r.p.m. After centrifugation at 1000 *g* for 15 min to remove nuclei and debris, ATPase activities were determined on the resulting supernatants by measuring rate of liberation of inorganic phosphate (Pi) from disodium ATP [11]. Incubation media were made up as described previously [3]. The final concentrations in mM for the constituents for the different ATPases were as follows: $Na^+/K^+$ ATPase, $MgCl_2$ 6, KCl 5, NaCl 100, EDTA 0.1, Tris–Cl buffer pH 7.4, 30; $Mg^{2+}$ ATPase, $MgCl_2$, 6, EDTA 0.1, Tris–Cl buffer pH 7.4 135, $Ca^{2+}/Mg^{2+}$ ATPase $MgCl_2$ 6, $CaCl_2$ 0.15, EDTA 0.1 Tris–Cl buffer pH 7.4 135. After pre-incubation for 5 min at 37° disodium ATP was added to each tube to give a final concentration of 3 mM. The sample blank containing no enzyme, standards and unknowns were incubated at 37° for 30 min. The reaction was stopped by putting the samples on ice. Inorganic phosphate was determined on 1 ml aliquots of the incubated mixtures by the addition of Lubrol-molybdate solution followed by vortexing and standing at ambient temperature for 10 min. Extinction at 390 nm was measured. All assays were done in triplicate and run with enzyme and reaction blanks. Samples were compared for phosphate content with standards of $KH_2PO_4$. Specific activities were calculated as nmol/Pi/hr/mg protein.

*Reagents used for determination of Pi.* Lubrol-molybdate solution was prepared according to Atkinson [11].

(a) Lubrol 5% was prepared monthly by warming in distilled water at 37°. Solutions became cloudy after prolonged storage due to micellar aggregation, but could be clarified by warming for 10–20 min at 37°.
(b) Acid molybdate $(NH_4)_2\ Mo_7O_{24}\ 4H_2O$ was 2% in 1.8M $H_2SO_4$ and was stored in dark bottles at 4°.
(c) Lubrol molybdate was prepared daily by mixing solution (b) 25 vol., solution (1) 10 vol. and water 65 vol. This reagent was stable for 8 hr at room temperature or for 24 hr at 4°.

Protein content was determined according to the method of Lowry [12] and bovine serum albumin used as a standard.

*Results and discussion.* The use of 60 mM LiCl/kg in the food produced plasma lithium concentrations comparable to therapeutic levels in humans, below the toxic level [13]. These can be seen in Table 1. Rats receiving lithium gained weight less rapidly than corresponding controls, but they did not lose weight and appeared reasonably healthy.

In the present experiments, in both iris and visual cortex, lithium treatment for 4 weeks produced an increase in $Mg^{2+}$ activated and $Ca^{2+}/Mg^{2+}$ activated ATPase activities. Enzyme activation was achieved much more rapidly in iris than in visual cortex; the increase which showed high significance ($P < 0.01$) being maintained for the rest of the medication. In the visual cortex, maximum activation of 45 per cent in $Mg^{2+}$ ATPase was not achieved until after 4 weeks, differences from control being significant at 3 and 4 weeks. A similar picture was found for $Ca^{2+}/Mg^{2+}$ ATPase with a lower degree of activation of about 23–24 per cent obtained after 2 weeks' administration in the iris (just significant, $P = 0.02$) and after 4 weeks ($P < 0.01$) in visual cortex. $Na^+/K^+$ ATPase activity was not significantly affected in either tissue (Figs. 1 and 2). These results agree in general with those of Hesketh *et al.* [2], where a relatively smaller but nevertheless significant increase in rat synaptic plasma membrane $Mg^{2+}$ ATPase activity was found after 3 weeks' dietary administration of lithium chloride (using the same conditions as the present experiments) amounting to 25 per cent. Hesketh *et al.* [2] also found an increase of 15 per cent in mitochondrial $Mg^{2+}$ ATPase activity after one week of lithium medication with no change in $Na^+/K^+$ ATPase activity.

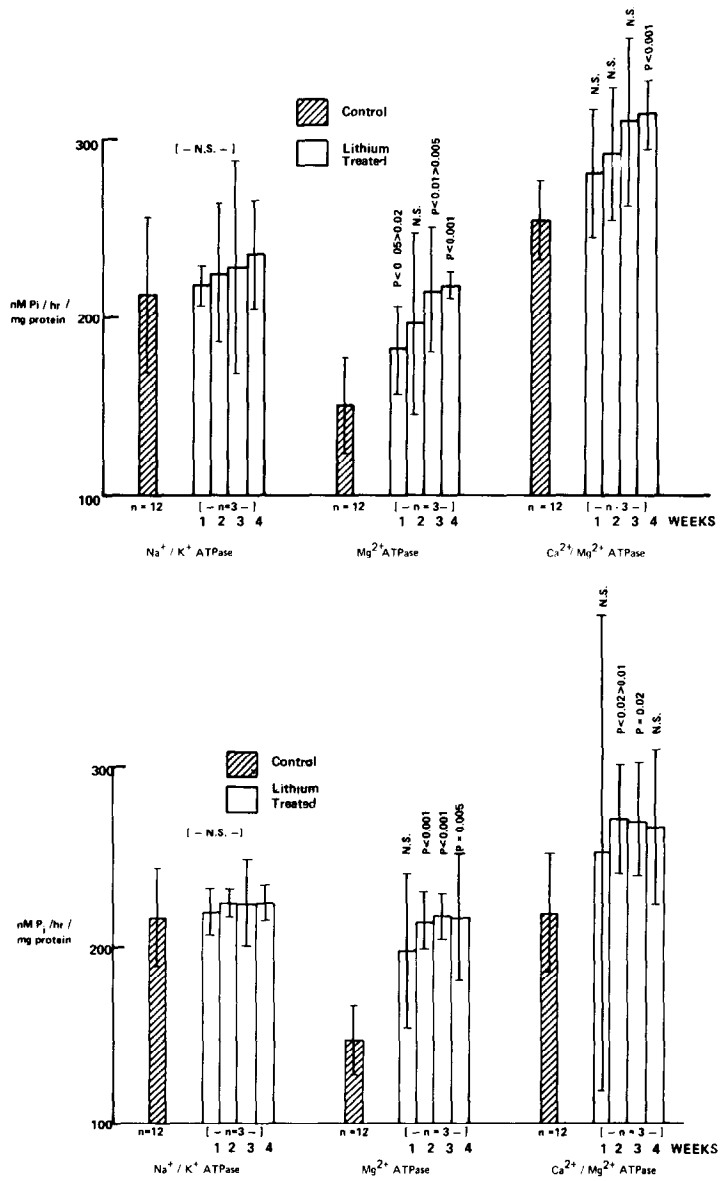
The present experiments reveal similarities between the relative activities of the different cation activated ATPases in iris, a peripheral tissue without neurones containing neuromuscular junctions and a central tissue such as visual cortex, loaded with neurones and inter-synaptic connections. It must be noted, however, that our preparation was a relatively crude homogenate and was not separated into subcellular fractions and that a proportion of $Ca^{2+}/Mg^{2+}$ ATPase and $Mg^{2+}$ ATPase could have arisen from muscle structures of iris.

The available data also underline the selectivity of action of lithium on the ATPases in both animal and human tissues. It can be concluded that the major effects of lithium administration in a variety of tissues appear to be confined to calcium and magnesium activated ATPases with very little action on sodium pump ATPase. Investigations using rat brain subcellular material have already been described

Table 1. Plasma lithium and body weights of rats during lithium administration

| Group | Plasma lithium (mM) Days of treatment | | | | Body weight (g) Days of treatment | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 7 | 14 | 21 | 28 | 0 | 7 | 14 | 21 | 28 |
| Control | 0 | 0 | 0 | 0 | 200.33 ± 10.37 (3) | 219.66 ± 11.26 (3) | 286.66 ± 2.36 (3) | 303.00 ± 19.00 (3) | 314.50 ± 5.50 (3) |
| Lithium | 0.65 ± 0.23 (3) | 0.54 ± 0.02 (3) | 0.73 ± 0.03 (3) | 0.58 ± 0.07 (3) | 192.00 ± 11.34 (3) | 197.67 ± 19.6 (3) | 201.66 ± 18.4 (3) | 230.00 ± 26.77 (3) | 233.00 ± 15.46 (3) |

The results are expressed as mean ± S.D. with the number of animals in parentheses.




Figs. 1 and 2. Specific activities of adenosine triphosphatases in rat visual cortex (Fig. 1) and in rat iris (Fig. 2), effect of dietary administration of lithium chloride (60 mM/kg diet). Number of rats shown in figures as "n". *P* values for significance of difference of each value from its corresponding control are shown where appropriate. N.S. = not significantly different.

[2] and a survey of erythrocyte membrane ATPases in patients with manic–depressive illness in comparison with normal control subjects [3] showed an increase in $Mg^{2+}$ ATPase activity of the order of 43 per cent after 2–4 weeks' treatment. This effect was undoubtedly due to lithium since it occurred irrespective of changes in clinical state.

The specificity of action of lithium on the calcium and magnesium activated ATPases and its rapidity of effect on the iris preparation may have some significance in assessing its toxic action and give further support to the view that the therapeutic action of lithium is connected with its ability either to substitute for calcium and magnesium in tissues, or to bring about changes in the distribution of these divalent cations [14]. Lithium intoxication is characterized by neuromuscular symptoms and signs, including general muscle weakness, ataxia and tremor of the hands, symptoms which do not respond to antiparkinsonian medication but may respond to β-adrenergic blocking drugs [15].

The effects of lithium on $Na^+/K^+$ ATPase, $Mg^{2+}$ ATPase and $Ca^{2+}/Mg^{2+}$ ATPase were investigated and compared in rat iris and visual cortex, following chronic dietary administration of lithium chloride 60 mM/kg diet, which achieved therapeutic levels without toxicity. Lithium medication produced activation of the order of 45 per cent in $Mg^{2+}$ ATPase and 23–24 per cent in $Ca^{2+}/Mg^{2+}$ ATPase in both tissues, but the effect was more rapidly achieved in iris. $Na^+/K^+$ activity was not significantly affected in either tissue. The results are discussed with respect to the therapeutic and toxic effects of lithium.

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# Exhalation of mercury—further evidence for an oxidation–reduction cycle in mammalian tissues

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According to the original observations of Nielsen-Kudsk [1,2], the hydrogen peroxide pathway appears to be responsible for the oxidation of inhaled mercury vapor ($Hg^0$) to divalent inorganic mercury ($Hg^{2+}$) in mammalian tissues [3,4]. The exhalation of volatile mercury from rats injected with mercuric chloride was the first evidence that $Hg^{2+}$ might be reduced to $Hg^0$ [5].

The ability of ethanol to depress retention of inhaled vapor [1] and to increase exhalation after exposure of animals to mercury vapor [6] might be accounted for on the basis that ethanol is a substrate in the peroxidatic action of catalase [7], and thus could compete for catalase with mercury. The increase in exhalation produced by ethanol in mice treated with mercuric chloride might be explained by inhibition of the oxidation of $Hg^0$ that has been produced by reduction of $Hg^{2+}$ [8]. The exhaled mercury was identified as $Hg^0$.

However, ethanol is known to have a wide variety of metabolic effects. For example, it might directly effect reduction of $Hg^{2+}$ by changing tissue NAD/NADH ratios. In order to test more specifically the role of catalase in the oxidation–reduction of $Hg^{2+}$, we examined the effects on exhalation of mercury of a well known inhibitor of catalase, 3-amino-1,2,4-triazole (AT). In addition, tests were made on a strain of mice having a genetic variant differing from the normal strain solely in catalase activity [9]. The tissue catalase activities in these animals have been published elsewhere [4].

An initial colony of $Cs^B$ mice (acatalasemic) [9] and $Cs^A$ mice (the corresponding wild type) were provided by Dr. Feinstein, Argonne National Laboratory, Argonne, Il. CBA/J mice were purchased from Jackson Laboratories, ME. Mice treated or not treated with AT were exposed to radioactive metallic mercury vapor in the chamber described previously [10]. AT was recrystallized from a commercial product according to the method described by Tephly *et al.* [11]. AT was injected, i.p. at a dose of 1 g/g body weight, 30 min prior to exposure or immediately after exposure. Exhalation rates were measured by collecting exhaled mercury in an absorbent* held in a tube attached to the outlet of a 500 ml plastic bottle containing the exposed mice. Air was passed through the bottle continuously at a flow rate of 4 liters/min. Radioactivity of the absorbent was counted once an hour by a Packard model 3002 Tri-Carb scintillation spectrometer. During the counting, the collection of air samples was interrupted for about 2 min.

The cumulative amount of exhaled mercury increased continously during the period of observation in all groups of animals (Fig. 1). To assess the contribution of volatilization of mercury from the fur and external surfaces of the mice, volatile mercury was recorded from dead animals (curve F). Volatile mercury from dead animals was always lower than from living animals, confirming previous observations that the main source of volatile mercury was exhalation [5].

In animals exposed to mercury vapor (upper figure), those having reduced catalase activities exhaled more mercury (curves A, B and C) than animals with normal catalase activity. Aminotriazole given 30 min before or after a single i.p. dose of mercuric chloride (lower figure) increased the amount of exhaled mercury. An important difference from the animals exposed to mercury vapor is the 1 to 1.5 hr time lag occurring before the effect of aminotriazole was observed. Another large difference is the much lower amount of mercury exhaled in the mercuric chloride-treated animals.

The increased exhalation cannot be explained by changes in tissue distribution produced by aminotriazole or acatalasemia. Lung deposition is actually reduced in vapor-exposed acatalasemic mice or normal mice pretreated with aminotriazole. Furthermore, aminotriazole given after vapor, or given before or after $HgCl_2$, produced no significant changes in tissue distribution, as evidenced by measurement of mercury in lung, blood, brain, liver, kid-

* Hopcalite (Hopkins & Williams, Ltd., Birmingham, England).