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IN VITRO EFFECTS OF LITHIUM CHLORIDE ON ATPases OF RABBIT CEREBRAL SYNAPTIC MEMBRANES

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Abstract—Lithium chloride (3 mM) stimulated Mg ATPase and inhibited ($Na^+ - K^+$)ATPase of rabbit cerebral synaptic membranes. The inhibitory effects of lithium were dependent on the ionic strength of the incubation medium. Lithium increased the activation energies of Mg ATPase and (Na^+–K^+)ATPase.

The observations that sodium transport may be altered in states of depression [1–3] has been linked to a reduction in the level of activity of plasma membrane (Na^+–K^+) ATPase [4,5]. The therapeutic effects of lithium salts have been correlated with the concentration of lithium in the red blood cells of the patients [1,2,4]. The optimum therapeutic concentration range of lithium in plasma is 0.6–1.5 mM [6]. Studies on the effects of lithium on brain amine levels suggest that there is an even better correlation between the therapeutic effects of the salts and the concentration of lithium in brain tissues [7].

Lithium accumulates in the particulate fraction of brain homogenates after longterm lithium treatment [8] such that, if we assume that there is no binding of the lithium, the intracellular concentration would be minimum of 100 per cent higher than the plasma concentration and 600 per cent higher than that of the CSF, which would require active transport of lithium into cells.

There is no direct evidence that the sodium pump transports lithium, although the work of Glen *et al.* [9] does suggest that lithium can stimulate the extrusion of sodium ion from red cells in low potassium solutions. These latter authors argue that lithium could have its therapeutic effects by stimulating the sodium pump of the nerve endings.

The majority of studies of the effects of lithium on ATPase activity have been of an acute nature [2–15]. In view of the longterm prophylactic effect of lithium [16], it may be that the important biochemical effects of lithium occur after prolonged lithium administration. However, it is apparent that the membrane effects of lithium reported on patient's receiving lithium treatment [17] are not completely reproducible in rat brain, which perhaps is due in part to the high renal clearance of lithium observed in rats [18]. Hesketh *et al.* [19] observed no *in vitro* effects with lithium (10^{-8} to 10^{-3} M) on rat cerebral synaptic membrane ATPase. However, in contrast, Ploegar [11] observed that lithium inhibited (Na^+–K^+)ATPase during supramaximal stimulation of non-myelinated rat vagii.

The failure of Hesketh *et al.* [19] to demonstrate *in vitro* effects with lithium might be related to the low levels of lithium which they used, or perhaps the effects of lithium are dependent on the specific activity of the ATPase. Noradrenaline and serotonin stimulate (Na^+–K^+) ATPase of rabbit cerebral synaptic membranes [20]. This paper reports the *in vitro* effects of lithium on the ATPase of rabbit cerebral synaptic membranes under conditions designed to manipulate the specific activity of the enzymes.

METHODS

Preparation of synaptic membranes. Albino male rabbits were stunned and decapitated. The brain was rapidly exposed and removed to ice-cold isotonic sucrose (0.32 M). A slice of cerebral cortex was removed, weighed and homogenized in 10% w:v ice-cold isotonic sucrose, by six thrusts of a power-driven teflon pestle (clearance 0.18 mm) at 900 r.p.m.

The homogenate was centrifuged at 1000 *g* for 20 min. The supernatant was recentrifuged (20,000 *g* for 30 min). The resultant pellet was resuspended in distilled water and recentrifuged (30,000 *g* for 30 min). The pellet was resuspended in distilled water and layered onto a discontinuous sucrose density gradient (15 ml of 1.2 M, 10 ml of 0.8 M) and centrifuged at 30,000 *g* for 2 hr. The synaptic membrane fraction was collected at the 0.8/1.2 M sucrose interphase. The membranes were washed twice with distilled water and ATPase activity was assayed within 12 hr of the animal's death.

Assay of ATPases. ATPases were assayed by determination of the rate of release of inorganic phosphate (Pi). Membranes were incubated at 37°, unless otherwise stated, in a 50 mM Histidine–HCl buffer, pH 7.4 (unless otherwise stated). Total ATPases activity was assayed in a medium containing 150 mM Na, 10 mM K, 3 mM Mg and 3 mM ATP, unless stated otherwise. The Mg ATPase was assayed in a medium containing 3 mM Mg and 3 mM ATP. All data presented here was obtained using Boehringer disodium ATP. The membranes were added to a final concentration of 100 μg/ml and the incubation was commenced by the addition of ATP.

Determination of Pi. The incubations were stopped by adding 4 ml of colour solution prepared by dissolving 10 g of ammonium molybdate and 10 g of Lubrol WX in 1 l. of 0.9 M H_2SO_4. The colour was

left to develop and was stable for 3 hr at room temperature. 10^{-4} M NA affects the estimation of Pi by this method. When possible this was overcome by using NA at concentrations not greater than 20 μM. However, if [NA] 20 μM were required standard Pi graphs were prepared for each [NA] used. The specific activities of the ATPase are measured in μmoles Pi liberated per mg of membrane protein per hr.

Protein estimation. Protein was estimated by the method of Lowry *et al.* (21).

RESULTS

The in vitro *effect of lithium on the activities of ATPases.* The effects of 3 mM lithium chloride on the activities of the total and Mg ATPases were compared with the effects of 3 mM Histidine–HCl buffer, pH 7.4. Lithium increased the activity of the Mg ATPase from 12.6 ± 0.9 to 15.7 ± 1.4 μmoles/mg of synaptic membrane protein/hr (mean ± S.D. of 9 experiments). The total ATPase was decreased by lithium ($P < 0.005$) and thus the value of the (NA^+–K^+) ATPase was reduced from 18.9 ± 1.9 to 10.0 ± 1.2 μmoles Pi/mg/hr. There was no Na-Mg ATPase (an ouabain insensitive ATPase) in rabbit synaptic membranes. The ouabain insensitive ATPase was identical to the Mg ATPase and was stimulated by lithium (12.3 ± 1.2 increased to 16.3 ± 1.4). The ouabain sensitive ATPase, i.e. difference between total and ouabain insensitive ATPase, was inhibited by lithium (19.3 ± 1.7 decreased to 10.7 ± 0.9). It is concluded that lithium does not stimulate (Na^+–K^+) ATPase of synaptic membranes, but inhibits this enzyme. Hesketh *et al.* (1977) observed no *in vitro* effects of 1 mM LiCl on ATPase of rat synaptic membranes. However, repeating these experiments using 1 mM LiCl, we observed qualitatively similar results. The specific activities of the enzymes used in the Hesketh *et al.* (1977) studies were much lower than the values obtained in these studies. To determine if the effect of lithium was dependent upon the specific activity of the enzyme, the effect of lithium on the submaximally stimulated (Na^+–K^+) ATPase was studied.

The effects of lithium on submaximally and maximally stimulated (Na^+–K^+) ATPase. The K_m for potassium was 1.8 mM. Synaptic membranes were incubated in Histidine–HCl buffer, pH 7.4, and containing 150 mM, Na^+; 5 mM, Mg^{2+} and 2 mM, K^+. The effects of 5 mM lithium were compared with the effects of 5 mM Histidine buffer, pH 7.4. In the absence of potassium there was no ouabain-sensitive ATPase activity and the total ATPase was not significantly greater than that of the Mg ATPase. Thus, we could not demonstrate the existence of the Na^+ ATPase described by Gilbert [6]. Lithium did not influence the activity of the (Na^+–K^+) ATPase at 0 mM K^+, and the percentage inhibition of the enzyme increased from 53 per cent at 2 mM [K^+] to 73 per cent at 10 mM [K^+] (Fig. 1).

The K_m for sodium was 80 mM. The effects of 5 mM lithium was compared with those of 5 mM Histidine–HCl, pH 7.4 (Fig. 1). At a sodium concentration of 100 mM there was no significant inhibition of the (Na^+–K^+) ATPase.

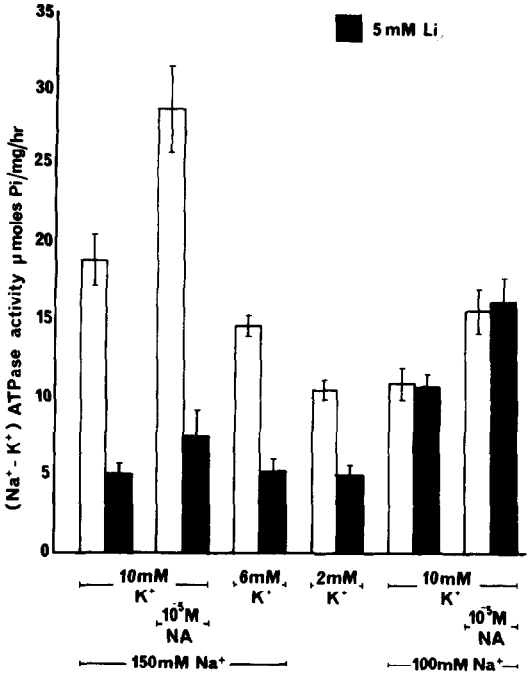

Fig. 1. Effects of 5 mM lithium chloride on (Na^+–K^+) ATPase. The influence of lithium on synaptic membrane ATPase was studied under varying concentrations of potassium and sodium ions and in the presence or absence of 10^{-5}M noradrenaline.

The inhibitory effects of lithium cannot be dependent on the specific activity of the enzyme. Lithium does not substitute for either sodium or potassium in the enzyme reactions, and further, the inhibitory effects of lithium cannot be overcome by increasing the concentration of either ion; an indication that lithium does not compete with these ions for binding to the enzyme. The marked inhibition of (Na^+–K^+) ATPase by lithium in 150 mM Na^+ medium compared with the negligible effect in 100 mM Na^+ medium suggests that the *in vitro* inhibitory effects of lithium may be related to the ionic strength of the medium.

The (Na^+K^+) ATPase is stimulated by NA and 5HT. Lithium (5 mM) decreased the (Na^+–K^+) ATPase in the presence of NA (10 μM). The percentage decrease in the enzyme activity was the same in the presence of the amine as in its absence (Fig. 1).

The effects of lithium on the activation energies of synaptic ATPases. The activites of both Mg ATPase and (Na^+–K^+) ATPase are increased with an increase in the incubation temperature to an optimum of 37°. At temperatures between 37 and 45° there was a marked decrease in the activity of the (NA^+–K^+) ATPase and of the Mg ATPase. The effects of lithium (5 mM) on the ATPases of cerebral synaptic membranes were studied at various temperatures in the range 20–45°. At temperatures below 30° there was not a significant difference in the values of the total and Mg ATPases in the presence of lithium. Thus it was not possible to measure the (Na^+–K^+) ATPase activity under these conditions.

However, at temperatures greater than 30°, the activity of the (Na^+–K^+) ATPase could be assayed in the presence of 5 mM lithium. The data were fitted to an Arrhenius plot. The activation energies were calculated from the slope of the linear part of the plot 20–37°. The activation energy of the (Na^+–K^+) ATPase was 2.7 kcal/mmole. Because of the absence of the (Na^+–K^+) ATPase in the presence of lithium at temperatures less than 30°, the activation energy was plotted for the data available for temperatures between 30 and 37°. The slope of the line was markedly increased and the activation energy was increased to 19 kcal/mmole. The activation energy of total ATPase was increased from 6.4 to 17 kcal/mmole and the activation energy of the Mg ATPase was increased from 5.9 to 16.4 in the temperature range 20–35°.

DISCUSSION

The discrepancies between the results obtained here and those from the *in vitro* study by Hesketh *et al.* [19] cannot be readily explained. However, it seems most likely that lithium's inhibitory effect on (Na^+–K^+) ATPase is dependent on the ionic strength of the medium. Hesketh *et al.* [19] assayed the (Na^+–K^+) ATPase in a medium containing 100 mM Na^+. These results also show that lithium is not competing with sodium or potassium for a site on the enzyme. During lithium therapy it is likely that the internal lithium concentration in nerve cells rises to 3 mM or greater [8]. The *in vitro* studies of Hesketh *et al.* would indicate that there is no induced change in ATPase activities during lithium therapy. Thus the longterm prophylactic effect of lithium [16] may be due to a delay in the accumulation of lithium by the nerve cells.

The results from this study are not consistent with the model of the therapeutic effects of lithium prosposed by Glen *et al.* [9]. The (Na^+–K^+) ATPase has been linked with a number of synaptic phenomena, including regulation of transmitter release [22]. Lithium has been reported to enhance transmitter release from the brain [23]. Recently it has been suggested that noradrenaline stimulation of (Na^+–K^+) ATPase was involved in a post-synaptic transmitter mechanism [24] and so we would expect lithium to block the post-synaptic effects of NA. This raises the question, does lithium have its therapeutic effects by inhibiting a sodium pump and thus enhancing the concentration of noradrenaline in the synaptic cleft or does it have the opposite effect of blocking the post-synaptic receptor activity? It could do both, since lithium has been shown to be a useful drug in controlling bipolar manic depression [25]. There are two problems here. At present, there is not a satisfactory biochemical model for manic depressive illnesses and secondly, there is not quite a suitable animal model for the study of the pharmacodynamics of lithium.

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