The stimulation of rat brain monoamine oxidase by dietary lithium chloride*

I. Dawood and W. Welch, Jr

Department of Biochemistry, University of Nevada, Reno (Nevada 89557, USA), 2 November 1978

Summary. Lithium chloride administered to rats in drinking water for 30 days caused an increase of whole brain monoamine oxidase specific activity to approximately 140% of control. Carboxylesterase and formyltetrahydrofolate synthetase activities were not affected by Li^+ either in vivo or in vitro.

Lithium salts (chloride or carbonate) have been used effectively as both treatment and prophylaxis for patients suffering from manic-depressive illness and recurrent depression[1-6] and appear to act synergistically with other drugs used in the treatment of depressive illness[7]. Results from many laboratories suggest that lithium treatment induces a change in the metabolism of the biogenic amines[8-11]. We report here that lithium causes an enhancement of brain mitochondrial monoamine oxidase (monoamine: oxygen oxidoreductase (deaminating) E.C.1.4.3.4, MAO).

Materials and methods. Rats used for this report were female, Sprague-Dawley line, purchased from Holtzman, Madison, Wisconsin. All rats were housed 2 per cage, in a single room at 26 °C with a 12-h light cycle (06.00-18.00 h) and allowed constant access to Purina lab chow and tap water. Control animals were maintained as above while the experimental rats were given lithium chloride in tap water (Fisher certified, 0.5 mg LiCl/ml). Rats were sacrificed by decapitation at approximately 10.00 h. Reagents were of the highest purity offered by commercial suppliers. Clorgyline (M&B 9302) was a gift from May & Baker Co. Tetrahydrofolic acid was produced by the method of Blakely[12] as modified by Samuel et al.[13]. The mitochondrial isolation procedures were variations of those described by Hawkins[14] and Gey and Pletscher[15]. The supernatant of the high speed centrifugation step of the above was used as a source of formyltetrahydrofolate synthetase and carboxylesterase.

Monoamine oxidase was assayed essentially as described by Green and Haughton[16]. The reaction was stopped after 30 min at 37 °C by adding 1 ml of 0.5 N acetic acid. Unreacted substrate did not affect color formation. Product formation was linear with time for at least 50 min and with protein concentration up to at least 4.6 mg. There was no evidence of substrate inhibition. The colorimetric assay was in close agreement with the manometric assay (Creasey[17]). Formyltetrahydrofolate synthetase was assayed by the method of Rabinowitz and Pricer[18] and carboxylesterase activity by the p-nitrophenyl propionate assay of Higgins and Lapides[19]. The serum collected immediately after decapitation was analyzed for lithium by atomic absorption spectroscopy at Washoe Medical Center. We thank Andrew Sohn and Nancy Cafereti for making these determinations possible. Protein concentrations were determined by a modified phenol procedure[18] using bovine serum albumin as a standard.

Results. Monoamine oxidase: In preliminary experiments with untreated rats, the specific activities of MAO were found to be in the same range of values reported elsewhere[20,21]. The K_m of the rat brain enzyme for tyramine is 0.26 mM. The rat liver enzyme had a K_m of 0.52 mM (tyramine) in reasonable agreement with other reports (0.3 mM, Green et al.[16], 0.85 mM, Oswald et al.[22]). Additionally, the assay was checked by use of the established MAO inhibitors iproniazide (8×10^{-5} M), pargyline (2.5×10^{-5} M) and M & B 9302 (clorgyline, 2×10^{-6} M). After 20 min preincubation (37 °C), the levels of inhibition (as percent of uninhibited control) were 50, 11 and 46 respectively. These values are within the ranges reported elsewhere[21,23-26].

The table reports data from a series of experiments in which rats of equal age were divided into 2 groups [an experimental (+) and control (−)] and tested for Li^+ stimulation. In general, the rats receiving lithium consumed approximately one half the amount of water consumed by the control rats and appeared to be more placid than the controls. Diarrhea was not observed. Average weight gains of both groups were essentially identical, consistant with the reports of Abreau and Abreau[27,28]. In experiments 1-3

Effect of dietary lithium on MAO activities of rat brain[a]

Experiment	Enzyme source	Days on Li^+	Concentration of Li^+ (mg/L)[b]	Activity (nmole/min/g brain)[c]	Specific activity (nmole/min/mg protein)[c]	Li^+ stimulation (% of control)[d]
1	Mitochondria	30	– –	11.6 (+) 8.4 (−)	1.11 (+) 0.79 (−)	140
2	Mitochondria	15	0.43 (+) 0.01 (−)	9.2 (+) 8.4 (−)	0.89 (+) 0.79 (−)	113
3	Mitochondria	30	0.54 (+) 0.00 (−)	11.0 (+) 9.0 (−)	1.39 (+) 0.96 (−)	145
4	Whole cell	15	0.26 (+) 0.04 (−)	37 (+) 36 (−)	0.54 (+) 0.48 (−)	114
5	Whole cell	30	0.30 (+) 0.00 (−)	40 (+) 32 (−)	0.56 (+) 0.42 (−)	135

[a] The (+) sign indicates LiCl (0.5 mg/ml) in drinking water. The (−) sign indicates no added lithium. Rats were sacrificed by decapitation and the brains of each experimental group were pooled for homogenization and assay. Monoamine oxidase activity was measured by the colorimetric method using tyramine (8 mM) as substrate. Initial weight of both experimental groups were within 5 g. Weight gains were identical for both groups. The rats in each experiment rats in both groups were the same age. Experiments 2 and 3 were initiated at the same time using rats of the same age. Experiments 4 and 5 were conducted in a similar fashion. [b] Serum levels at end of experiment. Equal aliquots from each animal were pooled for assay. [c] Specific activity of MAO in isolated mitochondria from pooled brains was determined in experiments 1-3. Specific activity of whole cell homogenates was determined in experiments 4 and 5 using the supernatant from a low speed centrifugation (600× g) of the brain homogenates. Each pool consisted of 4 animals. Details of isolation and assay may be found in the materials and methods section. The SD of the assays (4-6 determinations of each pool) were 1-2%. [d] Stimulation was calculated as 100× the ratio of the specific activity of the treated animals to that of the controls.

(table), MAO levels of purified mitochondria were measured after 15 or 30 days of lithium administration. As is the case with liver[14], much of the brain MAO activity remained in the supernatant fraction. Therefore, the apparent stimulation observed could be an artifact of the selection of MAO activity associated with the mitochondrial fraction. Experiments 4 and 5 were performed to estimate the changes in total cellular MAO. The amount of stimulation was similar and showed the same time dependence.

When assays of liver and brain mitochondrial monoamine oxidase were conducted in sodium, potassium or ammonium phosphate, tris acetate or tris hydrochloride (pH = 7.4, [anion] = 0.064 M), no statistically significant difference in reaction velocity was found. Lithium (0–22 mM) also had no effect on velocity as previously reported[29].

Formyltetrahydrofolate synthetase and carboxylesterase: Formyltetrahydrofolate synthetase (1.64 ± 0.07) and carboxylesterase (41 ± 1 nmoles/min/mg prot) specific activity was not affected by the presence of lithium chloride (up to 125 mM) in the assay mix. No difference could be found in the synthetase or esterase levels of lithium treated or control animals of the table.

Discussion. The data presented here indicate that oral lithium administration at (or somewhat below) the therapeutic lower limit[1,4,30] causes an increase in MAO activity of whole rat brain. A stimulation of platelet MAO was observed by Bockar et al.[31] in patients being treated with lithium. The average magnitude of the increase (148–150% of control) is similar to that reported herein for rat brain. Serum Li^+ concentrations were approximately 2-fold higher than those used in this study. The increase in MAO appears to be time dependent; increases were larger at 30 days than at 15 days of lithium feeding. The slow increase in MAO activity is consistent with the report of Rastogi and Singhal, who observed no significant change in MAO activity in rats treated with lithium after 6 days[46]. The lithium effect is indirect as the cation has no effect upon the catalytic event itself. A similar relationship was seen in lithium's effect on the transport of norepinephrine across nerve membranes[32].

Carboxylesterase and formyltetrahydrofolate synthetase levels were not affected by lithium either in vivo or in vitro in contrast to some other enzymes outside of the biogenic amine pathway[27,28,33–35]. The reported Li^+ inhibition of K^+ activated enzymes was not observed in the case of the synthetase.

Evaluation of the reported variation of MAO with estrous cycle[38–43] with respect to our results is difficult because of large differences in method and reported variations in brain regions[44]. It is significant that in all experiments Li^+ did not fail to increase MAO specific activity. The calculated probability of such a result is due to chance is low (0.02) assuming the wide variation reported to occur in the hypothalamus[38]. The consistancy of the observed stimulations (table) also argues that cyclic MAO variations are not a likely explanation of the results. More detailed investigations are now indicated.

It is interesting that the magnitude of the lithium-induced change in tryptophane hydroxylase (decrease[45]), norepinephrine uptake (increase[32]) and tyrosine hydroxylase (decrease[46]), is also approximately 30% of the control value. The relationships of these phenomena with the increased MAO activity observed herein is complex and require further study.

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