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Lithium effects on high-affinity tryptophan uptake: evidence against a stabilization mechanism

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Acute effects of lysergic acid diethylamide (LSD) and chlorimipramine on tryptophan uptake into rat brain synaptosomes were studied in rats treated chronically with dietary lithium and in control rats. The acute drug treatments did not change V_{max} or K_m for tryptophan uptake in control rats. In lithium-treated rats, chlorimipramine increased and LSD decreased V_{max}. These results indicate that, rather than stabilizing serotonin production through tryptophan uptake changes, chronic lithium treatment may increase the responsiveness of tryptophan uptake to changes in serotonin utilization.

Lithium ion is effective in the treatment and prophylaxis of affective disorders[16,26] and in the reduction of aggressive behaviors in man[25] and in animals[34,36]. While the mechanisms of these actions are unknown, it has been proposed that they are related to effects of lithium on serotonin[18,31] which is thought to be involved in the control of affect and aggression[4,18,28,31].

Since lithium is effective against both mania and depression, its effects may involve stabilization of some neurotransmitter system against endogenous or exogenous perturbations[7,33]. A possible model for behavioral stabilization in animals is provided by the effects of lithium on shock-elicited fighting in rats[27]. In addition to reducing shock-elicited fighting, lithium treatment has been shown to prevent both the increase in fighting after acute treatment with lysergic acid diethylamide (LSD)[35] and the decrease after acute chlorimipramine[26]. As acute LSD and chlorimipramine affect serotonin by contrasting mechanisms[1,9,11] the prevention by lithium of their effects on shock-elicited fighting may indicate that some of the behavioral effects of lithium arise from stabilization of serotonergic function.

A potential source of instability in serotonergic function is its susceptibility to changes in substrate availability[10,12]. The rate of serotonin synthesis may be limited by the rate of entry of tryptophan, the amino acid precursor of serotonin, into the cell[10]. Lithium treatment, in relatively high doses, has been shown to increase the high-affinity uptake of tryptophan into rat brain synaptosomes[20,21,24]. This has been proposed

to lead to decreased synthesis of tryptophan hydroxylase, the rate-limiting enzyme, decreasing the potential variability of serotonin synthesis[24]. The role of the tryptophan uptake system in behaviors or pharmacologic effects stabilized by lithium is not known.

The present study was undertaken in order to examine whether lithium stabilizes tryptophan transport against drug interactions in a manner analogous to its stabilization of shock-elicited fighting. Acute treatments with LSD or chlorimipramine were used as models for perturbations against which lithium exerts a stabilizing behavioral effect[27].

Male Sprague–Dawley rats weighing 160–180 g were fed a diet containing lithium as well as supplemental sodium and potassium[30]; an equal number of control rats received the same diet without the lithium. Lithium dosage was started at 20 mEq/kg dry diet, increased after one week to 40 mEq/kg dry diet, and increased after two weeks to 60 mEq/kg dry diet. Lithium treatment was continued at 60 mEq/kg dry diet until 5 weeks of treatment was completed. This was identical to the regimen that reversed LSD and chlorimipramine effects on shock-elicited fighting[27]. Under these conditions, there was no significant difference in weight between lithium-treated and control rats. Lithium-treated rats displayed polyuria and polydipsia but otherwise did not appear or act differently from controls. Serum lithium was 0.71 $\pm$ 0.07 (S.D.) mEq/l and brain lithium (corpus striatum) was 0.60 $\pm$ 0.12 mEq/kg.

Ten lithium-treated and 10 control rats were killed 15 min after i.p. injection of 5 mg/kg chlorimipramine hydrochloride (Geigy); 4 lithium-treated and 4 control rats received an equal vol. of normal saline. Ten lithium-treated and 10 control rats were killed 7 min after injection of 40 μg/kg LSD bitartrate in normal saline (Sandoz); 10 lithium-treated and 10 control rats received an equal vol. of normal saline. These doses and times correspond to the conditions under which optimal effects on shock-elicited fighting were previously observed[27].

Rats were killed by decapitation without anesthesia. Cerebral cortex, hippocampus, corpus striatum, and brain stem (pons and midbrain excluding colliculi) were dissected over ice using gross landmarks[22]. Tissue was homogenized in cold 0.32 M sucrose containing 1 mM EDTA and 25 mM Tris·HCl, pH 7.5. After centrifugation for 10 min at 700 $\times$ g, the pellet was washed and the combined supernates were centrifuged for 15 min at 17,000 $\times$ g. The resulting pellet was washed with the sucrose solution and used as crude synaptosomes[13]. Protein was determined by the method of Lowry et al. with bovine serum albumin as standard[23].

Tryptophan uptake[2,12,19,20] was measured within 45 min of homogenization[8]. About 200 μg of crude synaptosomal protein was incubated in 0.5 ml pre-warmed medium containing 100 mM NaCl, 5 mM KCl, 1.5 mM $MgCl_2$, 1.5 mM $CaCl_2$, 20 mM Tris·HCl at pH 7.5 and 20 mM D-glucose. After pre-incubation at 37 °C for 60 sec, L-tryptophan (Sigma Chemical) containing ^3H (side chain labeled, New England Nuclear, spec. act. 15–20 Ci/mmol) was added to give final concentrations of 5, 10, 20, 30, 40 and 50 μM. After incubation for 2 min, uptake was stopped by addition of 5 ml 0.32 M sucrose containing 10 mM Tris·HCl (pH 7.5) and 1 mM $CaCl_2$ (see ref. 2) and the samples were promptly centrifuged at 17,000 $\times$ g for 10 min. The supernate was

removed by aspiration, the insides of the tubes dried with cotton swabs, and the pellet was washed with the sucrose solution, taken up in NaOH, and the ^3H determined by liquid scintillation counting. Incubation was performed in duplicate; samples incubated at 4 °C were used as blanks[2].

Synaptosomes from several rats in the same treatment group were combined, as in previous studies[20]. Double reciprocal plots based on mean values from 3 separate experiments were used to calculate V_{max} and apparent K_m for each treatment group. Confidence limits for the parameters of the least squares regression lines were estimated by multiplying the S.E. of the estimate for the parameter by the two-tailed t value corresponding to the indicated level of statistical significance[38].

Table I shows the effect of chronic lithium treatment on the apparent maximal velocity of high-affinity tryptophan uptake. Consistent with the results of Knapp and Mandel[20], V_{max} was increased by lithium treatment in corpus striatum, hippocampus and brain stem. Lithium treatment had no effect in cerebral cortex. Lithium treatment did not significantly change apparent K_m in any of the regions.

Table II shows the effects of acute LSD and chlorimipramine treatment on tryptophan uptake in corpus striatum. Neither LSD nor chlorimipramine caused significant changes in control rats. By contrast, in lithium-treated rats, chlorimipramine increased and LSD decreased the V_{max} for tryptophan uptake. LSD treatment has been reported to decrease firing rate of, and serotonin efflux from, serotonin-containing cells in rats not treated with lithium[1,9,11]. By contrast, chlorimipramine was shown to acutely increase serotonin efflux[9] while similarly decreasing firing rate[1,11]. The changes in V_{max} in the lithium-treated rats after chlorimipramine or LSD were thus parallel to the acute changes in serotonin efflux, but not in firing rate, that have been observed after administration of these drugs in rats not treated with lithium.

Apparent K_m tended to change parallel to changes in V_{max}. This is consistent with the reported transport of tryptophan by an ordered exchange mechanism[17,37]. In the case of tryptophan transport, a number of other compounds probably share the transport system[6]. Changes in concentration of tryptophan or a congener could inhibit tryptophan uptake by competition, as has been reported with L-DOPA[8], or could increase uptake due to stimulation of exchange.

TABLE I

Effect of lithium treatment on the calculated V_{max} for high affinity tryptophan uptake in crude synaptosomes from rat brain

Region	*Control*	*Lithium*
Corpus striatum	3.60 ± 0.45*	6.95 ± 0.73**
Hippocampus	1.94 ± 0.06	2.39 ± 0.10**
Cortex	0.98 ± 0.08	0.90 ± 0.10
Brain stem	0.99 ± 0.02	1.34 ± 0.03**

* V_{max} is in nmol tryptophan/(mg prot/min) ± S.E.
** Lithium-treated different from control, $P < 0.05$.

TABLE II

Kinetic parameters for high affinity tryptophan uptake in crude synaptosomes from rat corpus striatum

V_{max} is in nmol tryptophan/(mg prot./min).

Acute treatment	*Control*	*Lithium*
None	3.6 ± 0.45	6.95 ± 0.73*
Chlorimipramine	2.98 ± 0.83	11.77 ± 1.42**
LSD	2.63 ± 0.06	2.49 ± 0.06***

* Li^+ different from control, $P < 0.05$.
** Li^+ and chlorimipramine different from Li^+ and from chlorimipramine, $P < 0.05$.
*** Li^+ and LSD different from Li^+, $P < 0.05$.

Changes similar to, but smaller than those shown for corpus striatum occurred in brain stem of lithium-treated rats treated with LSD and chlorimipramine. LSD and chlorimipramine did not change the parameters significantly in hippocampus or cerebral cortex of either control or lithium-treated rats.

While lithium treatment has been shown to render shock-elicited fighting less susceptible to drug-induced changes[26], the data presented here indicate that lithium had the opposite effect on tryptophan uptake. These data do not appear consistent with a model in which lithium treatment would stabilize serotonin production through increased tryptophan uptake. These effects were produced by a relatively low-dose regimen of lithium administration, with moderate tissue levels of lithium and only mild evidence of lithium side-effects.

Possible interpretations consistent with the data reported here include: (a) tryptophan uptake is not directly related to serotonin synthesis or to serotonin function; (b) the stabilization of shock-elicited fighting by lithium administration is not directly related to effects on serotonin; and (c) the behavioral stabilization by lithium results from facilitation of compensatory changes in serotonergic function.

The relationship between tryptophan uptake and serotonergic activity is not established, but some studies indicate that serotonin synthesis from tryptophan, which is limited by tryptophan availability[10], may be directly related to release of serotonin from serotonergic neurons. Tryptophan loading potentiated some effects of treatment with a monoamine oxidase inhibitor that appeared to be related to increased synthesis of serotonin[12]. Evidence has been presented that changes in tryptophan uptake account for the diurnal variation in brain serotonin content[14]. Hery et al. recently demonstrated that nerve stimulation increased the release of serotonin formed from recently infused tritiated tryptophan[15]. Because LSD and chlorimipramine did not change tryptophan uptake in control rats, these earlier results may indicate that while increased tryptophan availability may potentially increase serotonin production, increased utilization of serotonin may not necessarily stimulate tryptophan uptake.

Reports of synergistic clinical effects of lithium and i.v. chlorimipramine[29] and lithium and tryptophan[5] imply that the effects on tryptophan uptake observed in this report may be related to some clinical effects of lithium treatment. There is no

evidence, however, directly linking tryptophan uptake to shock-elicited fighting. Drugs including L-DOPA[8] and Δ9-tetrahydrocannabinol[18] have been reported to affect tryptophan uptake, but the role, if any, of tryptophan uptake in their actions is not known.

In this study, treatment with lithium, rather than stabilizing the rate of tryptophan uptake, apparently rendered it increasingly responsive to drug effects involving changes in utilization of serotonin. To the extent that tryptophan uptake is related to serotonin synthesis, it can be argued that if lithium treatment stabilizes behavior through effects on serotonin, it may do so by rendering serotonergic activity more flexible rather than more stable. This would be consistent with reports of augmentation of some serotonin effects by lithium administration[3,31,32].

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1 Aghajanian, G. K. and Wang, R. Y., Physiology and pharmacology of central serotonergic neurons. In M. D. Lipton, A. deMascio and K. F. Killam (Eds.), *Psychopharmacology: A Generation of Progress*, Raven Press, New York, 1978, pp. 171–183.

2 Bauman, A., Bourgoin, S., Benda, P., Glowinski, J. and Hamon, M., Characteristics of tryptophan accumulation by glial cells, *Brain Research*, 66 (1974) 253–263.

3 Buus Lassen, J., Potentiation of nialamide-induced hyperactivity in mice by acute and chronic treatment with lithium, L-tryptophan and the 5HT uptake inhibitor, FG 4963, *Acta physiol. scand.*, Suppl., 440 (1976) 106–110.

4 Chase, T. N. and Murphy, D. L., Serotonin and central nervous system functioning, *Ann. Rev. Pharmacol. Toxicol.*, 13 (1973) 181–197.

5 Chouinard, G., Jones, B. D., Young, S. N. and Arnable, L., Potentiation of lithium by tryptophan in a patient with bipolar illness, *Amer. J. Psychiat.*, 136 (1979) 719–720.

6 Christensen, H. N., *Biological Transport*, W. A. Benjamin, Reading, Mass., 1975, pp. 161, 176–189.

7 Davis, J. M. and Fann, W. E., Lithium, *Ann. Rev. Pharmacol. Toxicol.*, 11 (1971) 285–303.

8 Diez, J. A., Ward, W. G. and Summer, G. K., Tryptophan uptake in brain synaptosomes: effects of L-DOPA. *Brain Research*, 118 (1976) 534–539.

9 Farnebo, L.-O. and Hamberger, B., Drug-induced changes in the release of ^{3}H monoamines from field-stimulated brain slices, *Acta physiol. scand.*, 371 (1971) 35–44.

10 Forn, J., Tagliamonte, A., Tagliamonte, P. and Gessa, G. L., Stimulation by dibutyryl cyclic AMP of serotonin synthesis and tryptophan transport in brain slices, *Nature New Biol.*, 237 (1972) 245–247.

11 Gallager, D. W. and Aghajanian, G. K., Effects of chlorimipramine and lysergic acid diethylamide on efflux of precursor-formed ^{3}H serotonin: correlations with serotonergic impulse flow, *J. Pharmacol. exp. Ther.*, 193 (1975) 785–795.

12 Graham-Smith, D. G., Studies in vitro on the relationship between brain tryptophan, brain serotonin synthesis and hyperactivity in rats treated with a monomineacidase inhibitor and L-tryptophan, *J. Neurochem.*, 18 (1971) 1053–1066.

13 Gray, E. G. and Whittaker, V. P., The isolation of nerve endings from brain: an electron-microscopic study of cell fragments derived by homogenization and centrifugation, *J. Anat. (Lond.)*, 96 (1962) 79–88.

14 Hery, F., Rouer, E. and Glowinski, J., Daily variations of serotonin metabolism in the rat brain, *Brain Research*, 43 (1972) 495–465.

15 Hery, F., Simonet, G., Bourgoin, S., Soubrie, P., Artaud, F., Haman, M. and Glowinski, J., Effect of nerve activity on the in vivo release of [^{3}H]tryptophan in the caudate nucleus of the rat, *Brain Research*, 169 (1979) 317–334.
16 Hullin, R, P., McDonald, R. and Allsopp, M. N. E., Prophylactic lithium in recurrent affective illness, *Lancet*, i (1972) 1044–1046.
17 Inui, Y. and Christensen, H. N., Discrimination of single transport systems. The Na^+ sensitive transport of neutral amino acids in the Ehrlich cell, *J. gen. Physiol.*, 50 (1966) 203.
18 Iwata, H. Okamoto, H. and Kuramoto, I., The effect of lithium on serum tryptophan and brain serotonin in rats, *Jap. J. Pharmacol.*, 24 (1974) 235–240.
19 Johnson, K. M. and Dewey, W. L., Effect of △ 9-THC on the synaptosomal uptake of ^{3}H tryptophan and ^{3}H choline, *Pharmacology*, 17 (1978) 83–87.
20 Knapp, S. and Mandell, A. J., Short-term and long-term lithium administration: effects on the brain's serotonin biochemical systems, *Science*, 180 (1973) 675–677.
21 Knapp, S. and Mandell, A. J., Effects of lithium chloride on parameters of biosynthetic capacity for serotonin in the rat, *J. Pharmacol. exp. Ther.*, 193 (1975) 812–823.
22 König, J. F. and Klippel, R. A., *The Rat Brain, a Stereotaxic Atlas*. Williams and Wilkins, Baltimore, 1963.
23 Lowry, O. H., Rosebrough, N. J. Farr, A. L. and Randall, R. J. Protein measurement with the Folin phenol reagent, *J. biol. Chem.*, 193 (1951) 265–275.
24 Mandell, A. J. and Knapp, S., Regulation of serotonin biosynthesis in brain. Role of high affinity uptake of tryptophan into serotonergic neurons, *Fed. Proc.*, 36 (1977) 2142–2148.
25 Marini, J. L. and Sheard, M. H., Antiaggressive effect of lithium in man, *Acta psychiat. scand.*, 55 (1977) 269–286.
26 Marini, J. L. and Sheard, M. H., Thyrotropin-releasing hormone (TRH): lack of effect on shock-elicited fighting (SEF) in rats, *Commun. Psychopharmacol.*, 2 (1978) 139–144.
27 Marini, J. L., Sheard, M. H. and King, T., Role of brain serotonin system in lithium action, *Commun. Psychopharmacol.*, (1979) in press.
28 Murphy, D. L., Campbell, I. C. and Costa, J. L., The brain serotonergic system in affective disorders, *Progr. Neuropsychopharmacol.*, 2 (1978) 1–31.
29 O'Flanagan, P. M., Chlorimipramine infusion and lithium carbonate: a synergistic effect, *Lancet*, 2 (1973) 974.
30 Oleson, O. J., Jensen, J. and Thomsen, K., Effect of lithium on glucagon-stimulated cyclic AMP excretion in rats, *Acta Pharmacol. Toxicol.*, 35 (1974) 403–411.
31 Perez-Crouet, J., Tagliamonte, A., Tagliamonte, P. and Gessa, G. I., Stimulation of serotonin synthesis by lithium, *J. Pharmacol. exp. Ther.*, 178 (1971) 325–330.
32 Sangdee, C. and Franz, D. N., Lithium induced enhacement of 5-HT transmission at a central synapse, *Commun. Psychopharmacol.*, 2 (1978) 191–198.
33 Schou, M., Possible mechanisms of action of lithium salts. Approaches and perspectives, *Biochem. Soc. Trans.*, 1 (1973) 81–87.
34 Sheard, M. H., Effect of lithium on foot-shock aggression in cats, *Nature (Lond.)*, 228 (1970) 284–5.
35 Sheard, M. H., Astrachan, D. I. and Davis, M., The effect of D-lysergic acid diethylamide (LSD) upon shock-elicited fighting in rats, *Life Sci.*, 20 (1977) 427–430.
36 Smith, D. F., *Lithium and Animal Behavior*, Vol. 1., Eden Press, Montreal, 1977, pp. 13,27.
37 Westley, J., *Enzymic Catalysis*, Harper and Row, New York, 1969, pp. 93.
38 Winer, B. J., *Statistical Principles in Experimental Design*, 2nd edn. McGraw-Hill, New York, 1971, pp. 97, 139–145.