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LITHIUM TREATMENT AND TRYPTOPHAN TRANSPORT THROUGH THE BLOOD–BRAIN BARRIER

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Abstract—Tryptophan transport through the blood–brain barrier of lithium-treated animals did not differ from transport by controls. Lithium ion, itself, did not appear to alter such transport, nor did lithium treatment alter serum constituents so as to modify tryptophan passage through the blood–brain barrier. These results suggest that the accumulation of tryptophan in brain during lithium treatment probably results from the reported lithium-induced increase in the high-affinity, neuronal uptake system.

The lithium ion is an effective agent in the clinical treatment of manic depressive psychosis [1]. However, the mechanism by which this simple ion exerts its therapeutic action is still unclear although ionic [2], catecholaminergic [3] and indoleaminergic [4] mechanisms have been proposed.

The effect of lithium on serotonin metabolism is dose dependent and appears to change with the duration of treatment. Short-term treatment (3–5 days) increases brain serotonin turnover [5–7], brain tryptophan concentration [5], and the concentration of brain 5-hydroxyindoleacetic acid (5-HIAA) [5,7]. Upon continued treatment, blood tryptophan concentration returns to normal [8], but brain tryptophan [8,9] and 5-HIAA [10] remain elevated, and conversion of labeled tryptophan to serotonin and 5-HIAA [9] is accelerated. After 14–21 days of continuous treatment, serotonin turnover either returns to normal [11] or is reduced [12].

Because serotonin biosynthesis is substrate-sensitive [13], the increase in brain tryptophan concentration and indole metabolism during short-term lithium treatment could be due to the concomitant increase in blood tryptophan [6]. In order to account for the continued elevation in brain tryptophan at times when the blood tryptophan is normal, it has been suggested that chronic lithium treatment enhances tryptophan uptake into and transport within the brain [9]. Two transport systems are known to be important to serotonin biosynthesis. The first is the high-affinity uptake system thought to be primarily responsible for the transport of interstitial tryptophan into the neuron. Chronic lithium treatment has been shown both to increase the V_{max} of this system for tryptophan uptake and to concomitantly decrease tryptophan hydroxylase activity at nerve endings [14, 15], and some investigators [14,15] consider that these antipodal changes may underlie the mood stabilization produced by lithium therapy. A second transport system influencing serotonin biosynthesis is the neutral amino acid carrier of the blood–brain barrier. Lithium therapy could conceivably affect this system by a direct ionic effect on transport, by changes in the characteristics of the carrier system, or by altering the concentrations of blood constituents (e.g. other neutral amino acids or albumin) that affect tryptophan transport through this barrier system [16].

In the present study we have examined the effects of acute and chronic lithium treatment on the transport of tryptophan through the blood–brain barrier, the effects of lithium ions on such transport, and the influence of lithium administration on some serum constituents.

EXPERIMENTAL

Animals and treatments. Sprague–Dawley rats, either bred in our laboratory from Charles River stock (Charles River Labs, Wilmington, MA) or purchased from Mission Labs (Rosemead, CA), were maintained in an air-conditioned room on a 12:12 light–dark cycle for at least 3 days before use. Experimental animals were injected i.p. daily with LiCl at a dose of either 2 m-equiv./kg (Expt. 1) or 7.6 m-equiv./kg (Expt. 2) for 3 days, were injected twice daily with Li_2Co_3 (1.62 m-equiv./kg for 5 days (Expt. 3), or were fed a mash prepared from Purina rat chow (Ralston-Purina, St. Louis, MO) supplemented with 41 m-equiv. Li_2Co_3/kg of chow (Expt. 4). Control animals were either concurrently injected with saline or, in feeding experiments, fed lithium-free mash. The uptake of tryptophan through the blood–brain barrier was determined 3 hr after the last injection or in the early afternoon for animals fed the lithium diet. In some experiments, blood was collected from concurrently treated animals and serums were either pooled or separately analyzed for lithium or tryptophan, as indicated in the text.

Materials. L-[2-^{14}C] Tryptophan (54 Ci/mole) was obtained from Amersham-Searle (Arlington Heights, IL), L-[2,3-^{3}H]arginine (18 Ci/m-mole) and $^{3}H_2O$ (18 mCi/mole) from New England Nuclear (Boston, MA), and Hepes * buffer from CalBiochem (La Jolla, CA).

Methods. Brain uptake of tryptophan was determined by the method of Oldendorf [16]. Pentobarbital-anesthetized rats were injected via the common carotid artery with 0.2 ml of a solution containing 1.25 μCi

* Hepes = 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

Table 1. Effects of lithium treatment on tryptophan uptake into brain

Experiment*	Dose+ (m-equiv./day)	Days	BUI‡ Control	BUI‡ Experimental
1	2	3	39.1 ± 2.2 (4)	38.9 ± 2.3 (4)
2	7.5	3	37.7 ± 2.9 (12)	35.1 ± 3.2 (14)
3	3.2	5	40.0 ± 2.1 (3)	35.0 ± 7.5 (3)
4	0.4	21	38.1 ± 2.4 (5)	39.2 ± 3.6 (7)

* In Expts. 1 and 2, animals were injected i.p. daily with a lithium chloride solution. In Expt. 3, they were injected twice daily with a lithium carbonate solution and in Expt. 4 they were fed a mash containing 41 m-equiv. of lithium carbonate/kg of chow.

+ The daily dosage in Expt. 4 was calculated, assuming a daily consumption of 10 g of chow. Lithium levels of animals given the lithium-enriched diet were 0.65 ± 0.068 m-equiv./l of serum (N = 8) at the end of 2 weeks, and a value of 0.81 m-equiv./l was found for the pooled serum of animals killed at the end of week 3.

‡ The brain uptake index (BUI) is calculated as the ratio of $^{14}C/^{3}H$ in brain divided by $^{14}C/^{3}H$ in the injection mixture. The injection mixture consisted of 1.25 μCi [^{14}C]tryptophan, and 5 μCi $^{3}H_2O$, per 1 ml of 0.1 mM Hepes buffer, pH 7.4. Animals were killed 5 sec after injection of 0.2 ml of the isotope mixture into the carotid artery. Data are expressed as means ± S.E.M. (N).

[^{14}C]tryptophan and either 5.0 μCi $^{3}H_2O$, or 25 μCi [^{3}H]arginine, per ml of 0.1 mM Hepes buffer (pH 7.4). In some experiments, LiCl was added to the mixture. In other experiments, serum from control or experimental animals was added to the injection bolus after normalizing the serums to equivalent cold tryptophan concentration. Animals were decapitated 5 sec after injection and the right half of the brain rostral to the midbrain and minus the olfactory bulb was extruded through a 20-gauge needle and divided into two tubes containing 1.5 ml of Soluene-350 (Packard Instrument Co., Downers Grove, IL). The tissue was dissolved at 60° and suspended in Insta-gel (Packard) containing 4 ml of glacial acetic acid/liter, and then the $^{14}C/^{3}H$ ratio was determined. A portion of the injection mixture was also counted. The brain uptake index (BUI) was calculated as the ratio of isotopes in the tissue samples divided by the ratio in the injection mix [16].

Blood and brain tryptophan was determined by the method of Denckla and Dewey [17], with the exception that butyl acetate was used to extract the norharman from all samples. Lithium was estimated by flame photometry.

RESULTS

Table 1 shows that tryptophan uptake into brains of lithium-treated rats did not differ from controls, whether lithium was acutely injected daily or was chronically fed in the diet. Our observation of no lithium-induced alteration in transport was probably not due to inadequate or intermittent exposure of the capillary bed to circulating lithium. Animals given lithium-supplemented diets had lithium levels of 0.65 ± 0.07 m-equiv./l of serum (N = 8) at the end of week 2 of lithium feeding, and a value of 0.81 m-equiv./l was obtained in the pooled serum of the animals at the time of death at the end of week 3. These concentrations are within the therapeutic range for man.

The injection bolus used to measure uptake in these studies was lithium-free so as to permit examination of the barrier independently of ion effects on transport. In order to determine if the lithium ion itself altered tryptophan transport, varying concentrations of lithium chloride were introduced into the injection bolus. As seen in Table 2, these also were without effect even though the lithium concentration of the bolus exceeded the serum lithium concentration of rats consuming the lithium-mash diet by one to two orders of magnitude.

These results indicate that neither lithium treatment nor lithium ion exerts a direct effect on the neutral amino acid carrier. Our next question was whether lithium treatment might facilitate tryptophan transport into brain by altering the concentrations of key serum constituents such as the neutral amino acids, which compete with tryxtophan for transport, or the concentrations of albumin and free fatty acids, which alter the free/bound ratio of tryptophan in serum. To test this, pooled serum from controls, from animals acutely treated with lithium, or from animals fed a lithium-enriched diet for 3 weeks was incorporated into the injection bolus and the effect on uptake of tryptophan was measured. However, in order to avoid complications from unequal isotope dilution of the tracer by endogenous serum tryptophan, each serum pool, before incorporation into an injection mixture, was first analyzed for total tryptophan, and sufficient cold tryptophan was added to each to equalize the tryptophan

Table 2. Effects of lithium ions on tryptophan transport into brain

Li* (m-equiv./l)	BUI Control	BUI Experimental
20	36.9 ± 2.3 (3)	36.6 ± 1.7 (6)
200	35.3 ± 3.4 (3)	38.8 ± 2.3 (3)

* Two-tenths ml of a solution containing 1.25 μCi [^{14}C]tryptophan in a mixture of 0.1 mM Hepes buffer (pH 7.4) and sufficient LiCl to yield the concentrations of Li^+ indicated was injected into the carotid artery of one set of normal animals. A second set was injected with the same bolus without lithium addition. Data are expressed as means ± S.E.M. (N).

Table 3. Effects of serum from lithium-treated animals on tryptophan uptake into brain

Experiment*	BUI Controls	BUI Experimental
A	5.64 ± 0.26 (13)	5.65 ± 0.30 (9)
B	5.08 ± 0.50 (7)	5.71 ± 0.56 (7)

* Each ml of injection bolus contained 0.8 ml of serum from normal or experimental rats, 1.25 μCi [^{14}C]tryptophan, and 5 μCi ^3H_2O, in 10 mM Hepes buffer, pH 7.0. In A, control serum was derived from pooled serum from twenty untreated animals, and experimental serum from twelve animals killed 5 hr after i.p. administration of 7.5 m-equiv. Li^+/kg body weight. Before incorporation into the injectate both pools were analyzed for total tryptophan, and sufficient cold tryptophan was added to yield a final tryptophan concentration of 145 μM in both. In B, control serum was obtained from twenty normal rats, and experimental serum from twenty rats fed a lithium-supplemented diet (see text) for 21 days. Both pools were analyzed for tryptophan, and sufficient cold tryptophan was added to yield a final concentration of 166 μM in both. Data are expressed as means + S.E.M. (N).

content. As seen in Table 3, inclusion of sufficient serum to make up 80 per cent of the injection bolus greatly reduced the brain uptake index, presumably because of isotope dilution and competition by the other neutral amino acids in serum for carrier transport. However, controls and experimentals did not significantly differ in their uptake of tryptophan.

Finally, it was of interest to determine if amino acid carriers other than the neutral amino acid carrier were affected by lithium. Arginine is carried on a basic amino acid carrier and its uptake into brain is normally about 2/3 that of tryptophan [18]. By substitution of [^{3}H]arginine for tritiated water in the usual injection bolus containing [^{14}C]tryptophan, it was possible to compare directly the status of the basic and neutral amino acid carriers in animals treated with lithium chloride (318 mg/kg) for 3 days. After injection of a bolus containing 1.25 μCi [^{14}C]tryptophan, and 25 μCi [^{3}H]arginine, per ml of injection mixture, the uptake ratio was 91.9 ± 4.4 (N = 4) for controls and 88.9 ± 9.8 (N = 4) for experimentals, suggesting that neither system was affected by lithium treatment.

DISCUSSION

It seems generally agreed that lithium treatment increases brain tryptophan concentration, and that this increase is not dependent upon a corresponding increase in blood tryptophan. Our results indicate that such tryptophan accumulation in brain does not result from lithium-induced changes in the neutral amino acid carrier of the blood–brain barrier, nor is it an indirect effect of lithium on blood constituents which might influence tryptophan uptake from blood to brain. This increase in brain tryptophan following lithium treatment most probably results from increased "high-affinity" uptake of tryptophan into synaptosomes, which has been demonstrated by Mandell and Knapp [14] to occur after 5 days of lithium treatment, although other possibilities, such as alterations in protein synthesis or tryptophan efflux from the brain, need to be considered.

Our results also appear to indicate that tryptophan transport through the blood–brain barrier is unaffected by lithium ions either in blood or when added to the injection bolus. Two neutral amino acid transport systems which accept tryptophan as a substrate have been described in mammalian tissue. These, termed the L and A systems, were first defined in ascites cells, but seem to be common to many tissues [19]. The L system is sodium independent, is minimally responsive to pH, and accepts most of the neutral amino acids as substrates. The A system is more pH sensitive than the L system and transports all the neutral amino acids, but is sodium dependent, with amino acid and sodium transport linked in a 1:1 stoichiometry. Because lithium substitutes for or affects sodium concentrations in many systems [20], lithium would be expected to affect sodium-dependent transport of the A type. The fact that lithium did not alter tryptophan transport into brain suggests that the neutral amino acid carrier of the blood–brain barrier is an L-type system. This is in accord with reports that amino acid accumulation into brain slices [21] or into isolated brain capillaries [22] is sodium independent. More recently, however, isolated brain capillaries *in vitro* have been shown to accumulate the A system substrate, α-(methylamino)-isobutyric acid [23], and it has been suggested that both A and L systems may exist on the external capillary surface, in contact with brain, while the internal surface, in contact with blood, may consist exclusively of L-type carriers. It is, of course, possible that our *in vivo* studies showing that lithium does not alter tryptophan transport, as well as the other *in vivo* studies indicating sodium independence of neutral amino acid transport, may simply have been inadequate to detect ionic effects. For example, equilibrium between blood and the microbolus formed by subdivision of the injection bolus during its passage through the capillary plexus may be too rapid to expose the capillary cells sufficiently to ions affecting tryptophan uptake. It seems more likely, however, that the neutral amino acid carrier of the blood–brain barrier plays an insignificant role in the lithium-induced increase in brain tryptophan and that this accumulation results from lithium-induced changes in the high-affinity uptake system of brain cells.

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REFERENCES

1. L. Gerbino, M. Oleshansky and S. Gershon, in *Psycyhopharmacology: A Generation of Progress* (Eds. M. A. Lipton, A. DiMascio and K. F. Killam), p. 1261. Raven Press, New York (1978).
2. M. Eigen, *Neurosci. Res. Prog. Bull.* **14**, 142 (1976).
3. J. J. Schildkraut, S. M. Schanberg and I. J. Kopin, *Life Sci.* **5**, 1479 (1966).
4. S. Knapp and A. J. Mandell, *J. Pharmac. exp. Ther.* **193**, 812 (1975).
5. J. Perez-Cruet, A. Tagliamonte, P. Tagliamonte and G. L. Gessa, *J. Pharmac. exp. Ther.* **178**, 326 (1971).
6. A. Tagliamonte, P. Tagliamonte, J. Perez-Cruet, S. Stern and G. L. Gessa, *J. Pharmac. exp. Ther.* **177**, 475 (1971).
7. P. Poitou, F. Guerinot and C. Bohuon, *Psychopharmacologia* **38**, 75 (1974).

8. J. Schubert, *Psychopharmacologia* **32**, 301 (1973).
9. R. B. Rastogi and R. L. Singhal, *J. Pharmac. exp. Ther.* **201**, 92 (1977).
10. K. J. Collard and M. H. T. Roberts, *Neuropharmacology* **16**, 671 (1977).
11. E. L. Bliss and J. Ailion, *Brain Res.* **24**, 305 (1970).
12. A. K. S. Ho, H. H. Loh, F. Craves, R. J. Hitzemann and S. Gershon, *Eur. J. Pharmac.* **10**, 72 (1970).
13. P. A. Friedman, A. H. Kappelman and S. Kaufman, *J. biol. Chem.* **247**, 4165 (1972).
14. A. J. Mandell and S. Knapp, *Fedn Proc.* **36**, 2142 (1977).
15. S. Knapp and A. J. Mandell, *Science* **180**, 645 (1973).
16. W. H. Oldendorf, *Am. J. Physiol.* **221**, 1629 (1971).
17. W. D. Denckla and H. K. Dewey, *J. Lab. clin. Med.* **69**, 160 (1967).
18. W. H. Oldendorf, *Proc. Soc. exp. Biol. Med.* **136**, 385 (1971).
19. H. N. Christensen, *Adv. Enzymol.* **32**, 1 (1969).
20. A. I. M. Glen, M. W. B. Bradbury and J. Wilson, *Nature, Lond.* **239**, 399 (1972).
21. R. K. Margolis and A. Lajtha, *Biochim. biophys. Acta* **163**, 374 (1968).
22. H. Sershen and A. Lajtha, *Expl Neurol.* **53**, 465 (1976).
23. A. L. Betz and G. W. Goldstein, *Science* **202**, 225 (1978).