Short- and Long-Term Lithium Administration: Effects on the Brain's Serotonergic Biosynthetic Systems

Abstract. *Short-term treatment with lithium chloride stimulates the uptake of tryptophan and its conversion to serotonin by striate synaptosomes. Preincubation of striate synaptosomes with* L-*tryptophan and in vivo administration of* L-*tryptophan appear to act in a similar manner. Midbrain tryptophan hydroxylase activity is reduced in temporal continuity with the lithium-induced activation of tryptophan uptake and conversion. By 10 days, conversion of tryptophan to serotonin in nerve endings becomes a joint function of the maintained increased uptake of tryptophan and a decreased level of tryptophan hydroxylase activity in nerve endings. The occurrence of this delayed alteration corresponds in time to the previously described axoplasmic flow rate for tryptophan hydroxylase.*

The lithium ion is effective in the treatment of mania (*1*), in the prophylaxis of manic and depressive states (*2*), and in the modification of experimentally induced aggression (*3*). Hypothetical explanations advanced to account for these effects include ion-induced alterations in membrane excitability (*4*), retardant action on the mechanism of release of nerve ending biogenic amines (*5*), facilitation of catecholamine uptake by brain synaptosomes (*6*), and alterations in serotonin biosynthesis as reflected by measures of turnover applied to whole brain or to regions (*7*). Some have attributed augmented serotonin biosynthesis to a lithium-induced increase in concentrations of tryptophan in blood and brain (*8*), and this imputation may well correlate with studies demonstrating the antimanic effects of administration of L-tryptophan, the serotonin precursor (*9*).

Earlier work demonstrating a relation between concentrations of tryptophan and serotonin in the brain following tryptophan administration (*10*) makes the mechanism credible. Our recent work has demonstrated another possible regulatory influence in the biosynthesis of serotonin: a high-affinity synaptosomal uptake system ($K_m = 5.5 \times 10^{-5} M$) which is energy-dependent, temperature-sensitive, and drug-alterable (*11*). In contrast, the low-affinity tryptophan uptake system ($K_m = 3.3 \times 10^{-3} M$) appears not to be drug-sensitive (*11*). This report describes the effects of short- and long-term administration of lithium in vivo and of lithium chloride in vitro on uptake of tryptophan and conversion to serotonin by synaptosomes and on soluble tryptophan hydroxylase activity. Repeated administration of lithium chloride for 5 days appears to increase biosynthesis of serotonin in synaptosomes by stimulating the high-affinity uptake system for its precursor, tryptophan. Measurable tryptophan hydroxylase activity of the cell body is concomitantly reduced. Daily lithium treatment for 10 to 21 days results in the return to normal of synaptosomal biosynthesis, while the cell body tryptophan hydroxylase activity remains significantly below control levels. This decrease may be compensatory.

Adult male Sprague-Dawley rats were injected subcutaneously with 5 or 10 meq of either lithium chloride or sodium chloride per kilogram of body weight for 5, 10, or 21 days, or with 25 mg of tryptophan per kilogram 3 hours before they were killed (15 animals per group). The animals treated with lithium and sodium were decapitated 24 hours after the last drug injection. Two regions representative of serotonergic cell bodies and nerve endings, midbrain and striate, respectively (*12*), were dissected free and assayed for tryptophan hydroxylase activity according to our modification (*11*) of the methods of Ichiyama *et al.* (*13*). L-[1-^{14}C]Tryptophan (12 μc/μmole) was used as substrate. When the animals were killed blood was collected for determination of serum lithium by means of the method of Zettner *et al.* (*14*).

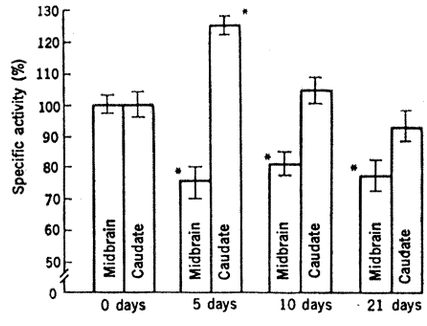

Fig. 1. The effects of daily (5, 10, or 21 days) lithium treatment on rat midbrain tryptophan hydroxylase activity and on rat striate synaptosomal conversion of tryptophan to serotonin. The controls treated with sodium chloride are represented by "0 days" treatment. Control midbrain enzyme activity: 100 pmole mg^{-1} hr^{-1}. Control striate conversion activity: 130 pmole mg^{-1} hr^{-1}. There were 15 animals per group. As early as 3 days (data not shown) after initiation of treatment, both enzyme and conversion activities significantly changed ($P = .02$) from their control levels. By 10 days, synaptosomal conversion activity had returned to control levels and remained there. The decrease in midbrain tryptophan hydroxylase activity persisted throughout these experiments ($P = .02$). Levels of statistical significance were determined by the Mann-Whitney U test (*21*). Markers on the bars represent the standard error of the mean. Asterisks indicate that the difference between these values and the control values was statistically significant.

The uptake of substrate by synaptosomes was studied by using L-[3-^{14}C]-tryptophan (29 μc/μmole) as the substrate. The striate 12,000*g* pellet, obtained from a 1,000*g* supernatant, was the source of serotonin synaptosomes. The incubation medium was 0.1*M* tris (*p*H 8.1) or Ringer phosphate (*p*H 7.5) containing 10 m*M* glucose and physiological concentrations of sodium and potassium. Uptake was linear for the 5-minute incubation. Substrate levels were varied from 10^{-5} to $5 \times 10^{-3} M$ in a total volume of 700 μl containing 0.2 to 0.3 mg of protein. Synaptosomes were isolated on Millipore filter paper (pore size, 0.65 μm) with a Millipore multiple-sample vacuum manifold. This synaptosomal preparation demonstrated two K_m's with regard to the uptake of tryptophan: a high-affinity K_m of $5 \times 10^{-5} M$ and a low-affinity K_m of $3 \times 10^{-3} M$. Passive diffusion was controlled by 5-minute incubations at 4°C. Hypotonic lysis of synaptosomes abolished all uptake of L-[3-^{14}C]tryptophan.

Altered tryptophan hydroxylase activity of nerve endings (intrasynaptosomal) or differential rates of efflux, or both, may influence the rate constant for uptake. However, we have observed that incubation of synaptosomes with labeled substrate for over 15 minutes leaves more than 90 percent of intrasynaptosomal tryptophan unconverted, which suggests that enzyme activity may not be relevant to experiments that have short incubation periods. Furthermore, in previous studies drugs have altered the rate of conversion independent of their effect on the rate of uptake (*11*). It is not clear whether accrued radioactivity represents net increases in total intrasynaptosomal substrate and product

or whether it represents a facilitated exchange of exogenous and endogenous tryptophan (*15*).

Figure 1 summarizes the effects of the daily administration of lithium chloride on midbrain-soluble and striate-synaptosomal tryptophan hydroxylase activity after 5, 10, or 21 days of treatment. The mean concentration of lithium in serum 24 hours after the last lithium dose was 0.6 ± 0.1 meq/liter. By day 5 synaptosomal conversion of tryptophan to serotonin was significantly elevated above control levels, and the midbrain enzyme was significantly reduced. By day 10 the synaptosomal conversion rate was no longer significantly augmented, and by day 21 it was slightly (but not significantly) below control levels of conversion. Throughout these experiments, tryptophan hydroxylase activity in the midbrain was significantly reduced below control levels. Following preincubation with lithium chloride in vitro, the effects observed after short-term lithium treatment were seen on the synaptosomal conversion rate but not on the soluble enzyme activity. This led to the speculation that a mechanism related to structural integrity (such as transport of substrate across intact membranes) is involved in the early response to daily lithium injections.

We have previously shown (*11*) that there are two synaptosomal uptake systems for tryptophan, with K_m's of $5.5 \times 10^{-5}M$ and $3.3 \times 10^{-3}M$, respectively, and that the system with the higher affinity for substrate is drug-sensitive. Figure 2 summarizes a kinetic study of the two synaptosomal transport systems for tryptophan with regard to substrate after daily administration of lithium chloride for 5 days. Lithium treatment augmented the high-affinity uptake system, but it did not produce a measurable effect on the low-affinity system. Facilitation of substrate uptake was seen also after preincubation of striate synaptosomes with lithium chloride in vitro.

In light of recent reports of the antimanic effect of tryptophan (*9*), it was of interest to us to determine if tryptophan, like lithium, stimulates synaptosomal uptake or conversion of L-[^{14}C]tryptophan, or both. Preincubation of striate synaptosomes with tryptophan ($10^{-4}M$) activates the high-affinity but not the low-affinity uptake system of tryptophan in a manner similar to that seen after preincubation with lithium chloride and after short- and long-term treatment with lithium chloride (Fig. 2). In addition, administration of tryptophan (25 mg/kg) 3 hours before the animals were killed increased the striate synaptosomal conversion of tryptophan to serotonin.

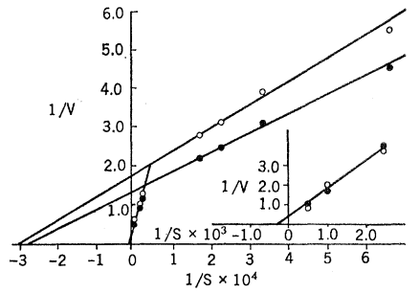

Fig. 2. The effect of daily lithium chloride treatment for 5, 10, or 21 days on two tryptophan uptake systems by rat striate synaptosomes, expressed as accumulation of [3-^{14}C]tryptophan. Substrate concentrations ranged from $1 \times 10^{-5}M$ to $2 \times 10^{-3}M$. Velocity (V) is expressed as picomoles $\times 10^3$ of L-[3-^{14}C]-tryptophan retained per milligram per 5 minutes; $1/V$ and $1/S$ represent the reciprocal values of velocity and substrate as they are defined. These data are the results of two separate experiments in which each experimental point was performed in duplicate. There is a V_{max} augmentation of 30 percent in regard to the high-affinity ($K_m = 3 \times 10^{-5}M$) tryptophan uptake system after pretreatment with lithium. There is no measurable change in either the apparent or calculated (inset) V_{max} of the low-affinity ($K_m = 3 \times 10^{-3}M$) tryptophan uptake system. Control (sodium chloride), ○——○; lithium, ●——●.

Studies (of varying durations), in which determinations of whole brain concentrations of serotonin or its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were used, have failed to demonstrate a lithium-induced effect (*7*, *16*). However, results from experiments in which either regional levels or various indices of turnover were used (*7*, *8*) for relatively short periods of time (5 days or less) are consistent with our hypothesis that lithium may initially stimulate serotonin biosynthesis in nerve endings. Recent interpretations of these studies emphasize the role of the concentrations of tryptophan in the brain because those levels ($4 \times 10^{-5}M$) were found to be below the K_m of tryptophan hydroxylase ($5 \times 10^{-4}M$) when the artificial pteridine cofactor 2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine ($DMPH_4$) is used for kinetic determinations (*17*). The argument has been made that since the enzyme was normally unsaturated (that is, the substrate was limiting) increases in substrate concentration produced by drugs or tryptophan administration and mass action kinetics were responsible for increased serotonin biosynthesis. However, a recent study by Friedman *et al.* (*18*), in which the natural pteridine cofactor was used, revealed a K_m for substrate of $5 \times 10^{-5}M$, which corresponds to brain concentrations of tryptophan. This suggests (i) that, in the natural state, tryptophan hydroxylase may not be so unsaturated as previously believed and (ii) that tryptophan hydroxylase activity and substrate *availability* are important regulatory factors.

In contrast to our finding of transiently increased biosynthesis of serotonin in nerve endings after 3 to 5 days of treatment with lithium chloride, we observed a concomitant, significant decrease in soluble (cell body) tryptophan hydroxylase activity, which persisted for the duration of the study (21 days). This is consistent with the report of Ho *et al.* (*7*), who showed a decrease of serotonin biosynthesis in the brainstem area after 28 days of lithium treatment.

The sequence we observed would be consistent with a feedback reduction in cell body enzyme activity and with the slow axoplasmic flow (of reduced enzyme activity) to the striate serotonergic nerve endings (*11*). Feedback regulation of a neurotransmitter biosynthetic enzyme has also been reported in our studies of drug effects on the activity of brain tyrosine hydroxylase (*19*).

Our data suggest that the therapeutic efficacy of lithium chloride in the treatment of mania is due in part to the compensatory decrease in brain tryptophan hydroxylase following the initial activation of uptake of tryptophan in nerve endings and conversion to serotonin. The latency of the clinical efficacy in manic states (10 to 14 days) (*1*) as well as the finding by Goodwin *et al.* (*20*) of decreased 5-HIAA in cerebrospinal fluid following long-term administration of lithium chloride in man is consistent with this hypothesis. In addition, we speculate that lithium could be potentiated in its antimanic effects by doses of L-tryptophan, since these treatments have in common the ability to initially facilitate the biosynthesis of serotonin in nerve endings.

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15. Synaptosomes from rats treated with lithium chloride and sodium chloride were preincubated for 5 minutes with L-[3-^{14}C]tryptophan ($1 \times 10^{-5} M$), after which the relative efflux rates of radioactivity were studied for up to 30 minutes. The measurable efflux rate of L-[3-^{14}C]tryptophan from the synaptosomal preparations of lithium-treated rats was the same as that from sodium-treated controls. The high-affinity, synaptosomal tryptophan uptake and conversion rates were augmented by pretreatment with lithium.
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22. Supported by National Institute of Mental Health grant MH 14360 and by the Friends of Psychiatric Research of San Diego, Inc. We thank Arnetta McPherson and Debbie Glockner for technical assistance.

17 November 1972; revised 22 January 1973 ■

Pronase Effect on Pancreatic Beta Cell Secretion and Morphology

Abstract. *Pronase at low concentration (4 micrograms per milliliter) produces a reversible increase of glucose-stimulated insulin release in isolated islets of Langerhans. Pronase also affects the ultrastructure of the beta cells by inducing extensive development of tight junctions as well as the accumulation of secretory product within the extracellular spaces.*

Proteolytic enzymes have been widely employed in the study of the molecular architecture and function of the cell membrane in various tissues. Incubation of intact cells with trypsin or Pronase has been reported to induce changes ranging from destruction of the cell membrane to subtle unmasking of previously unaccessible receptor sites located within the plasma membrane (*1*).

We have studied the effects of proteolysis on the pancreatic beta cells by incubating isolated rat islets of Langerhans with Pronase (*2*) at low concentration and observing concomitantly the morphology of the cells and the insulin release at low and high glucose concentration. The results show that such proteolytic treatment causes a reversible increase of the insulin response to glucose stimulation and peculiar ultrastructural alterations. These alterations include extracellular sequestration of secretory product and the appearance of extensive tight junctions (*3*) between islet cells.

Isolated islets were obtained by collagenase digestion of pancreases (*4*) removed from fed male albino rats. The incubation medium consisted of Krebs-Ringer bicarbonate buffer (KRB) equilibrated with 94 percent O_2 and 6 percent CO_2, and supplemented by 1 percent bovine serum albumin and, unless otherwise mentioned, 50 mg of glucose per 100 ml.

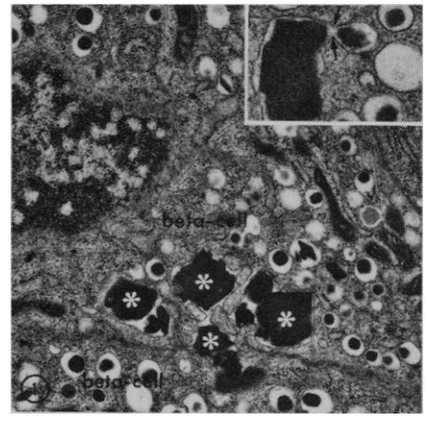

Fig. 1. Pronase-treated islet exposed to high glucose concentration (300 mg/100 ml). Several masses of electron dense material (asterisks) are seen within the intercellular space between two beta cells. The ultrastructure of the cellular organelles appears normal (× 11,600). The inset shows a secretory granule undergoing exocytosis (arrows) in proximity to a dense mass. The frequency of such images suggests that the dense masses are formed by coalescence of extruded granule cores (× 20,400).

In all experiments, the islets were divided in two pools and first incubated for 90 minutes at 37°C, either in 5 ml of KRB alone for the control pool or with Pronase E (*5*) at a concentration of 4 μg/ml.

After this first incubation period, both pools of islets were thoroughly washed in KRB. Groups of five islets were distributed in 1.5 ml of KRB containing glucose (either 50 or 300 mg/100 ml) and incubated for 90 minutes at 37°C. Portions of incubation media for measurement of immunoreactive insulin (IRI) content (*6*) were taken at 15, 30, 60, and 90 minutes.

Pelleted islets were briefly fixed in 2 percent phosphate-buffered glutaraldehyde and processed either for conventional thin-section electron microscopy or for freeze-etching by the method of Moor and Mühlethaler (*7*). Release of IRI from Pronase-treated islets incubated in a medium containing 50 mg of glucose per 100 ml did not differ significantly from that of the controls during the first 60 minutes of incubation (Table 1). After 90 minutes, however, IRI release by the Pronase-treated islets was approximately twice that of the controls. In a medium containing 300 mg of glucose per 100 ml, a twofold augmentation in the insulin response of Pronase-treated islets was evident after 30 minutes (Table 1).

In order to determine whether the enhancing effect of Pronase on glucose-induced IRI release was a reversible phenomenon rather than an irreversible leak of intracellular constituents, Pronase-treated islets were first incubated for 60 minutes in a medium containing 300 mg of glucose per 100 ml and then transferred to a medium with 50 mg of glucose per 100 ml and incubated for 60 minutes. Production of IRI, which was 41.4 ± 1.7 ng per milliliter per five islets (mean ± standard error of mean) after incubation at high glucose concentration, declined to a near basal level of 6 ± 0.3 ng per milliliter per five islets ($P < .001$), virtually identical to that observed in control islets.

In thin sections of Pronase-treated