23. Accumulation of Inositol 1, 4, 5-Trisphosphate in the Rat Brain as a Result of Long-term Administration of Lithium

By Yoshio ISHIMA^{*)} and Keizo WAKU^{**)}

(Communicated by Setsuro EBASHI, M. J. A., Sept. 12, 1991)

Abstract: Three-week-old male rats were given 2.7 mEq Li_2CO_3 / kg / day orally for 1 or 3 weeks, and the level of IP_3 in the brain was estimated. Although the level of IP_3 was not affected by the administration of Li_2CO_3 , IP_3 was further fractionated to I-1,3,4- P_3 and I-1,4,5- P_3 by HPLC. The relative percentages of I-1,3,4- P_3 and I-1,4,5- P_3 were 95.8 and 4.2 in controls and 69.6 and 30.4 in brains of rats treated with lithium. Since I-1,4,5- P_3 is known to be a messenger inside the cells to regulate cell-to-cell communication compared with I-1,3,4- P_3 , this increase in the relative level of I-1,4,5- P_3 in the brain might be relevant for the treatment of manic-depressive disorders.

Key words: Lithium; inositol 1,4,5-trisphosphate (I-1,4,5- P_3); rat brain; IP_3 ; long-term administration; affective disorders.

Introduction. Lithium has been used for the treatment of patients with affective disorders and other mental illnesses, and it has been proved empirically to be a very effective drug for these patients (Suzuki, 1982; Bunney *et al.*, 1987). However, the mechanism of the pharmacological impact of this cation on such patients remains obscure. We have now, for the first time, found material evidence that provides a clue to the therapeutic mechanism of action of lithium in the brain. In the present paper, experiments are described in which the effects of lithium are examined on the metabolism of IP_3 in the rat brain after 3 weeks of oral administration.

Methods. Chronic administration of lithium. Three-week-old male Sprague-Dawley rats (8 rats per group) were treated orally using a pipet with 2.7 mEq Li_2CO_3 / kg / day for one or three weeks. Twenty-four hours after the administration of lithium was terminated, the rats were decapitated, and the brains were removed immediately and cooled in ice-cold isotonic saline solution. Each brain (1.5 to 2.0 g) was weighed and homogenized in 4 ml of 5% (w / v) trichloroacetic acid for measurement of levels of inositol phosphates. A control group of 8 rats was fed the same diet without lithium treatment.

Analysis of IPs. The suspensions of homogenized brains in 5% trichloroacetic acid were centrifuged for 5 min at $1000 \times g$. Aliquots (1 ml) of the supernatant solutions were supplemented with 185 Bq of [^3H]IP, [^3H]IP₂, and [^3H]IP₃ ([^3H]IP₄ was also added in the case of fractionation by HPLC). Then the samples were extracted three times with 5 ml each of diethylether to remove the trichloroacetic acid.

Separation of IPs by ion-exchange chromatography. The samples were diluted with 5 ml of water for fractionation on Dowex-1-8X ion-exchange columns, as described by Downs and Michell (1981). Aliquots of each fraction were mixed with a scintillation-fluid cocktail [Triton X-100 / toluene (1 : 2, v / v) that contained 8.6 g of DPO and 0.26 g of POPOP per

^{*)} Neuropsychiatry, Ibaraki Prefectural Hospital, Tomobe, Ibaraki 109-17, Japan.

^{**)} Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Kanagawa 199-01, Japan.

2.4 l of Triton-toluene] for counting of radioactivity. The remainder of each fraction was lyophilized and fractionated by HPLC.

Fractionation by HPLC. The lyophilized samples that contained IPs were diluted up to 1 ml and one third of each solution was injected into the HPLC system. A precolumn of silicic acid (4 mm i.d. $\times$ 250 mm) (mounted before the injector loop), a Partisil SAX guard column (4.6 mm i.d. $\times$ 50 mm) and a Partisil SAX HPLC separating column (4.6 mm i.d. $\times$ 250 mm) were used as described by Wilson *et al.* (1985) (Cobert Associates, St. Louis, MO, USA).

Digestion of IPs. After lyophilization, the dried residues were digested with alkaline phosphatase (from bovine intestinal mucosa; Type 7-N; Sigma, St. Louis, MO, USA) as described by Rittenhouse *et al.* (1985).

Derivatization and gas chromatography. The resulting inositol samples were dried under a vacuum and 10 nmoles of manitol were added as an internal standard. Then half the solution was removed for counting of radioactivity to check the recovery, and the remainder was dried and the air above the sample was replaced by N_2 . At this step, the recovery of [3H]inositol was 40–60%. Then 100 μ l of a mixture of dry pyridine, trimethylchlorosilane and hexamethyldisilane (5 : 2 : 1, v / v) were added to each sample. The tubes were then capped and heated for 30 min at 55–60°C. Then 6 ml of water and chloroform (1 : 1, v / v) were added to each sample to extract the pyridine with water. After the water phase had been removed, the chloroform phase was dried under a stream of N_2 . The derivatives were redissolved in 2 μ l of hexane and 1 μ l was injected at 260°C onto a 25-m capillary fused-silica CBP-5 column (Shimazu Co, Japan) under He gas (column temperature, 210°C). Final results were calculated by considering the separate recoveries of xylitol, manitol, and [3H]inositol.

Lithium analysis. Concentrations of lithium in blood serum and tissues were determined by a modification of the atomic absorption spectrophotometric method used for determining levels of lithium in blood serum (Levy *et al.*, 1970). The atomic absorption spectrophotometer used was the Model 703 (from Perkin-Elmer Corp. Norwalk, CT, USA) with either a single-slot burner or a three-slot burner.

Results. The level of lithium in rat brain after oral administration of 2.7 mEq / kg /

Fig. 1. The effects of lithium on the levels of inositol phosphates in rat brain. Open bars, control; shaded bars, Li-test. Levels of IP, IP₂, and IP₃ were determined in homogenates of rat brain as described in the text. The varied control levels of IP in 1 week and 3 weeks seemed to be the differences in the course of development in 4 weeks and 6 weeks old rats. Results are means $\pm$ S.E. from 32 rats.

* $P < 0.01$, significantly different from the control by chi-square test.

Fig. 2. The effects of 3-week oral administration of lithium on the composition of IP₃.

Dotted bars, I-1,3,4-P₃; shaded bars, I-1,4,5-P₃. Details are described in the text. Results are means $\pm$ S.E. from 24 rats.

* $P < 0.01$, significantly different from the control by chi-square test.

day was about 0.25 mEq / kg of tissue. This result is almost the same as that of Sherman *et al.* (1981). The level of lithium in the serum was about 0.10 mEq / l throughout the experiment.

Fig. 1 shows the results of analysis of the levels of IPs in brains of lithium-fed rats compared with those in normal rats. After 1 week of continuous administration of lithium, the level of IP increased from 109 nmole / g in controls to 187 nmole / g (1.7-fold) and after 3 weeks the value rose from 199 to 483 nmole / g (2.4-fold) as also shown by Sherman *et al.* Levels of IP₂ and IP₃ in the rat brains showed no changes throughout the experiment.

We fractionated the IP₃ fraction into inositol 1,3,4-trisphosphate (I-1,3,4-P₃) and inositol 1,4,5-trisphosphate (I-1,4,5-P₃) by high-performance liquid chromatography (HPLC). I-1,4,5-P₃ is believed to be a potentially very important mediator of the control of cell-to-cell communication in the brain, acting as a second messenger inside the nerve cells (Berridge, 1987).

Fig. 2 shows the results for the brains of chronically lithium-treated rats compared with those for normal rats. The relative level of I-1,4,5-P₃ increased from 4.2% in the control to 30.4% as a result of 3 weeks of continuous administration of lithium, i.e. a 7.2-fold increase over levels in normally fed rats.

Discussion. Our results are the first to show that long-term administration of lithium increased relative levels of I-1,4,5-P₃ and decreased those of I-1,3,4-P₃ in the rat brain, while the total amount of IP₃ was unchanged. This result shows that lithium may inhibit the metabolism of IP₃ from I-1,4,5-P₃ to I-1,3,4-P₃. According to the experimental results of Batty and Nahorski, I-1,4,5-P₃ kinase may be inhibited by lithium in the rat cerebral cortex (Batty *et al.*, 1987), because the level of IP₄ was significantly reduced by incubation in 5 mM of lithium of slices of rat cortex. Therefore, the inhibition of I-1,4,5-P₃ kinase by lithium might explain the accumulation of I-1,4,5-P₃ after the chronic administration of lithium. Accumulation of the I-1,4,5-P₃ inside nerve cells causes the release of calcium ions from the endoplasmic reticulum of the cells into the inside of the axon terminals (Majerus *et al.*, 1988) which, in turn, causes the release of neurotransmitters contained in the synaptic vesicles of the nerve ending to the synaptic gaps (Miledi, 1973). On the other hand I-1,3,4-P₃ has much weaker ability (ca. 1 / 30) to mobilize the calcium ions from intracellular stores than I-1,4,5-P₃ (Irvine *et al.*, 1986).

Worley *et al.* (1987) published autoradiograms of rat brain showing high affinity,

selective binding sites for I-1,4,5-P₃ at a level 100–300 times higher than in other tissues. Moreover, they clarified the heterogeneous distribution of the receptors for I-1,4,5-P₃ in rat brain. These results together may provide information relevant to elucidation of action mechanisms of lithium on patients with affective disorders.

Acknowledgment. The authors wish to express their sincere gratitude to prof. Michio Toru of Neuropsychiatry, Tokyo Medical and Dental University, for reading our manuscript and offering invaluable advice and critique on our work.

References

- 1) Batty, I., and Nahorski, S. R. (1987): *Biochem. J.*, **247**, 797–800.
- 2) Berridge, M. J. (1987): *Ann. Rev. Biochem.*, **56**, 159–193.
- 3) Bunney, Jr., W. E., and Garland-Bunney, B. L. (1987): *Psychopharmacology*. Raven Press, New York, pp. 553–565.
- 4) Downes, C. P., and Michell, R. H. (1981): *Biochem. J.*, **198**, 133–140.
- 5) Irvine, R. F. *et al.*, (1986): *ibid.*, **240**, 301–304.
- 6) Levy, A. L., and Katz, E. M. (1970): *Clinical Chemistry*, **16**, 840–842.
- 7) Majerus, P. W. *et al.* (1988): *J. Biol. Chem.*, **263**, 3051–3054.
- 8) Miledi, R. (1973): *Proc. R. Soc. Lond. B.*, **183**, 421–425.
- 9) Rittenhouse, S. E., and Sasson, J. P. (1985): *J. Biol. Chem.*, **260**, 8657–8660.
- 10) Sherman, W. R. *et al.* (1981): *J. Neurochem.*, **36**, 1947–1951.
- 11) Suzuki, M. (1982): *Tokyo Jikeikai Medical J.*, **97**, 1–24.
- 12) Wilson, D. B. *et al.* (1985): *J. Biol. Chem.*, **260**, 13496–13501.
- 13) Worley, P. F. *et al.* (1987): *Nature*, **325**, 159–161.