INCREASED BRAIN MYO-INOSITOL 1-PHOSPHATE IN LITHIUM-TREATED RATS

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Received June 2,1976

SUMMARY

Lithium was found to produce a marked elevation in the levels of myo-inositol 1-phosphate in the cerebral cortex of treated rats. This effect was completely inhibited by atropine. A 40% reduction in the levels of myo-inositol 1-phosphate was observed when atropine was given alone.

INTRODUCTION

Lithium causes a reduction in the concentration of myo-inositol in rat cerebral cortex and this effect is inhibited by atropine and scopolamine (1,2). To further study these findings we have begun to look for changes in the levels of compounds metabolically related to myo-inositol and have found a striking increase in myo-inositol 1-phosphate in the brains of lithium-treated rats. In addition we have observed that this effect of lithium is also inhibited by atropine.

MATERIALS AND METHODS

Male Holtzman rats weighing 250 to 300 g were used in all experiments. Drugs were dissolved in deionized water (LiCl and NaCl, 0.75 M; atropine sulfate, 50 mg/ml) and were injected subcutaneously. At various time intervals after treatment the animals were decapitated and the heads were frozen immediately in liquid nitrogen. Doses and times of sacrifice are presented in the Results section.

myo-Inositol 1-phosphate and myo-inositol were measured as their trimethylsilyl (TMS) derivatives by gas-liquid chromatography. The samples were prepared by directly silylating portions of lyophilized frontal cerebral cortex (2 to 4 mg, dry wt.) with a mixture of pyridine: N,O-bis (trimethylsilyl) trifluoroacetamide: trimethylchlorosilane (10:2:1, v/v) for

24 hr at room temperature. The optimal ratio of the weight of dried brain to the volume of silylating solution was found to be 20 to 30 µg/µl. The samples were analyzed using a Varian Aerograph 2100 gas chromatograph equipped with hydrogen flame ionization detectors. Aliquots (6 µl) of the samples were injected onto 1.8 m x 4 mm i.d. columns. myo-Inositol 1-phosphate levels were measured using 3% OV-17 and myo-inositol determinations were made on 3% SE-30. myo-Inositol could not be analyzed using OV-17 because on this column TMS myo-inositol and TMS ascorbic acid have identical retention times and myo-inositol 1-phosphate could not be measured using SE-30 because of a similar interference from TMS glucose 6-phosphate. Pretested column packing material was obtained from Applied Science Laboratories, State College, Pa. All liquid phases had been coated on 80/100 mesh Gas Chrom Q. The oven temperature was isothermal at 180° and the carrier gas was helium with a flow of 90 cc/min. Quantitation was performed with the use of external standards injected before and after four or five tissue samples and linearity was examined at the beginning and end of each day. In order to obtain satisfactory results in terms of reproducibility and linearity it was necessary to prime the columns with five or six 10 µl injections of brain samples at the start of each day before proceeding with the analyses.

Mass spectra were obtained with a computerized LKB-9000 combined gas chromatograph-mass spectrometer. The operating conditions were as follows: ionization potential, 70 eV; source temperature, 250°; separator temperature, 250°; column 3% OV-17; helium flow, 30 cc/min.

Lithium levels were measured by flame photometry as previously described (2).

Student's t-test was used for the statistical analyses.

myo-Inositol 1-phosphate was a gift from Dr. D. E. Kiely, University of Alabama in Birmingham. It had been synthesized by the method of Kiely et al. (3).

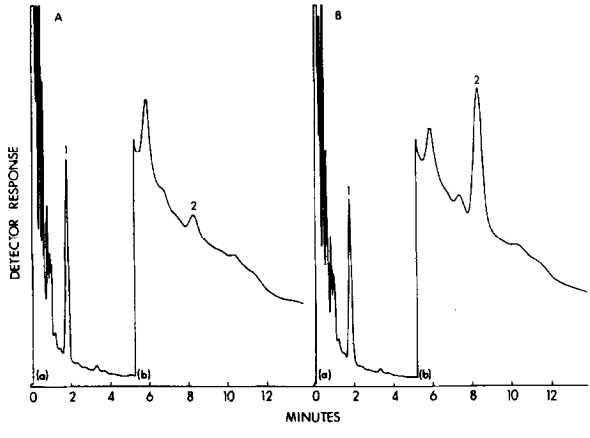

Figure 1. Gas chromatograms of trimethylsilylated derivatives from rat cerebral cortex: A, untreated; B, lithium-treated (5 meq/kg, sacrificed at 5 hr.) Peaks: (1) myo-inositol plus ascorbic acid; (2) myo-inositol 1-phosphate. Column: 3% OV-17 on 80/100 mesh Gas-Chrom Q. Sensitivity settings: (a) 256 X 10^{-11} amps full scale; (b) 128 X 10^{-12} amps full scale. Other chromatographic conditions are given in the text.

TABLE I

COMPARISON OF THE MASS SPECTRA OF AUTHENTIC TMS MYO-INOSITOL 1-PHOSPHATE AND THE UNKNOWN SUBSTANCE IN BRAINS OF LITHIUM-TREATED AND UNTREATED RATS

	Percent Relative Abundance											
m/e	318	387	389	419	432	433	470	507	560	569	659	749*
TMS myo-inositol 1-phosphate	100	38.5	19.9	4.1	8.5	10.1	5.9	3.2	1.8	1.8	2.7	12.5
Lithium-treated brain	100	40.3	18.1	3.5	9.9	10.2	4.3	2.4	1.7	0.9	2.6	12.4
Untreated brain	100	35.7	20.1	4.0	8.5	9.8	6.6	3.5	1.4	1.5	3.2	13.3

*M-15. The molecular ion is not observed in TMS inositol phosphate spectra obtained under the conditions used.

RESULTS

Gas chromatograms of brain samples from lithium-treated rats contained a markedly elevated peak in the region of the hexose phosphates (Fig. 1, peak 2) and this peak was found to have the same retention time as authentic TMS myo-inositol 1-phosphate (0.76 relative to TMS α-glucose 6-phosphate on OV-17). Retention times identical to the authentic standard were also observed on QF-1 and OV-225 (0.61 and 0.55, respectively, relative to TMS α-glucose 6-phosphate). Quantitation of this peak from both lithium-treated and untreated brain samples was performed on all three columns and the respective values in each group agreed to within 10%, indicating that the peaks from both groups were pure.

Further confirmation of the identity of this compound was obtained from mass spectrometric analysis. Representative ions are shown in Table I and indicate that the unknown in lithium-treated and untreated brain samples has a spectrum identical, within experimental error, to that of authentic TMS myo-inositol 1-phosphate. The data in Table I represent relative intensities of the ions obtained by setting the values for ion m/e 318 to 100%. The absolute intensities of all of the ions in the spectrum of the peak from the lithium-treated brain sample were increased approximately 10 fold when compared with those from the untreated sample, which corresponded to the degree of the elevation observed by gas-chromatographic analysis of these samples.

As shown in Table II LiCl at doses of 5 meq/kg and 1 meq/kg produced 9 fold and 2 fold increases, respectively, in the level of myo-inositol 1-phosphate in brain. In other experiments (results not shown) it was found that a 10 meq/kg dose of LiCl produced 18 to 22 fold elevations. NaCl had no effect on myo-inositol 1-phosphate. myo-Inositol levels were reduced by 19% in animals receiving a dose of 5 meq/kg of lithium. At 1 meq/kg an 8% decrease was observed but this difference was not statistically significant.

Since atropine and scopolamine inhibit the effect of lithium on myo-inositol (2) we conducted an experiment to see if atropine would alter the

TABLE II

EFFECTS OF LITHIUM ON MYO-INOSITOL 1-PHOSPHATE AND MYO-INOSITOL IN RAT CEREBRAL CORTEX

Treatment	myo-Inositol 1-Phosphate (mmoles/kg dry wt.)	myo-Inositol	Lithium (meq/kg dry wt.)
Untreated	0.339 ± 0.011	26.3 ± 0.86	---
NaCl (5 meq/kg)	0.317 ± 0.013	25.9 ± 0.62	---
LiCl (5 meq/kg)	2.94 ± 0.11 *	21.4 ± 1.03 **	6.88 ± 0.16
LiCl (1 meq/kg)	0.647 ± 0.043 *	24.2 ± 0.83	2.50 ± 0.14

Each value represents the mean ±SEM of 4 rats. The animals were killed 5 hours after treatment.

*Significantly greater than untreated group ($p<0.01$).
**Significantly less than untreated group ($p<0.02$).

TABLE III

EFFECTS OF LITHIUM AND ATROPINE ON MYO-INOSITOL 1-PHOSPHATE IN RAT CEREBRAL CORTEX

Treatment	myo-Inositol 1-Phosphate (mmoles/kg dry wt.)	Lithium (meq/kg dry wt.)
Untreated	0.345 ± 0.030	---
LiCl (5 meq/kg)	0.881 ± 0.096 *	3.87 ± 0.14
LiCl (5 meq/kg) plus Atropine (100 mg/kg)	0.315 ± 0.018 **	3.98 ± 0.32
Atropine (100 mg/kg)	0.207 ± 0.003 ***	---

Each value represents the mean ±SEM of 4 rats. The animals were killed 1 hour after receiving LiCl and 50 min after receiving atropine.

*Significantly greater than untreated group ($p<0.01$).
**Significantly less than LiCl group ($p<0.01$).
***Significantly less than untreated group ($p<0.01$).

effect of lithium on myo-inositol 1-phosphate. The results of this experiment are shown in Table III. At one hour after an injection of LiCl (5 meq/kg) a 2.5 fold increase was observed in myo-inositol 1-phosphate. This rise was completely inhibited by an injection of atropine sulfate. In addition it was found that atropine alone caused a 40% reduction in the levels of myo-inositol 1-phosphate. As we observed previously (2) atropine did not affect the concentrations of lithium in the brains of treated animals.

DISCUSSION

myo-Inositol 1-phosphate is an intermediate in the biosynthesis of myo-inositol and also is a product of phosphatidylinositol catabolism. The former is the L-isomer and the latter the D-isomer (4,5). (Nomenclature of these isomers was reversed in 1967, reference 6.) Since enantiomers do not separate by gas-liquid chromatography and since they have identical mass spectra our present data do not allow us to determine which of these compounds is the one affected. Lithium could cause an elevation of the L-isomer by inhibiting the phosphatase which converts myo-inositol 1-phosphate to myo-inositol in the synthetic pathway. Such an effect might result in a rise in levels of the substrate and a decrease in levels of the product which would be consistent with our findings. It has been reported that lithium (250 mM) inhibits this enzyme in mammary gland *in vitro* (7). However, whether lithium inhibits the brain phosphatase *in vivo* remains to be determined. An elevation of the D-isomer of myo-inositol 1-phosphate must also be considered in view of the following observations. Cholinergic agonists increase the turnover of phosphatidylinositol in brain (8-11) and one product of this reaction is thought to be D-myo-inositol 1-phosphate. Thus far there have been no reports describing a change in the *in vivo* levels of this product following cholinergic stimulation. However, in preliminary experiments we have recently found that pilocarpine and physostigmine produce a 2 to 3 fold elevation of myo-inositol 1-phosphate in brain. Secondly, the stimulated turnover of

phosphatidylinositol is blocked by atropine (8,10,11). And thirdly, it has been reported that lithium stimulates cholinoceptive neurons (12). Therefore, it seems reasonable to postulate that lithium increases the levels of myo-inositol 1-phosphate in brain by stimulating the breakdown of phosphatidyl-inositol. We are currently testing this hypothesis by examining the effect of lithium on phosphatidylinositol levels and turnover and we are also attempting to directly identify which enantiomer of myo-inositol 1-phosphate is affected by lithium.

ACKNOWLEDGEMENTS

This work was supported by U.S. Public Health Service Grants DA 00259, DA 4RG008, NS 05159 and RR00954.

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