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High-Dose Peripheral Inositol Raises Brain Inositol Levels and Reverses Behavioral Effects of Inositol Depletion by Lithium

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AGAM, G., Y. SHAPIRO, Y. BERSUDSKY, O. KOFMAN AND R. H. BELMAKER. *High-dose peripheral inositol raises brain inositol levels and reverses behavioral effects of inositol depletion by lithium.* PHARMACOL BIOCHEM BEHAV 49(2) 341-343, 1994.—Lithium (Li) reduces brain inositol levels. Berridge has suggested that this effect is related to Li's mechanism of action. It had previously been shown that pilocarpine causes a limbic seizure syndrome in lithium treated rats, and that these lithium-pilocarpine seizures are reversible by intracerebroventricular inositol administration to rats. We now show that although inositol passes the blood-brain barrier poorly, large doses of intraperitoneal (IP) inositol can also reverse Li-pilocarpine seizures. Using gas chromatography, IP inositol can raise brain inositol levels. Demonstration that inositol enters brain after peripheral administration provides a basis for possible pharmacological intervention in psychiatric disorders at the level of second messengers linked to the phosphatidylinositol cycle.

Lithium Inositol Li-pilocarpine seizures Blood-brain barrier

INOSITOL is a key intermediate in the phosphatidylinositol (PI) cycle. The latter serves as a second messenger system for numerous brain neurotransmitters (4). Inositol may be rate limiting in this cycle in the brain (8). Lithium reduces brain inositol levels (1) and affects behavior (11). Intracerebroventricular (ICV) replenishment of lithium-induced depletion of inositol reverses lithium's effects on behavior (10,12). The fact that inositol crosses the blood-brain barrier poorly (19) has limited behavioral studies of inositol given peripherally (13). The present study shows that inositol, given at high enough doses peripherally, enters the brain and raises brain inositol levels significantly, as measured both chemically and behaviorally.

Li-pilocarpine seizures are induced by pilocarpine after pretreatment 24 h before by low doses of LiCl (9). These Li-pilocarpine seizures can be prevented by ICV administration of myo-inositol but not by L-chiro-inositol, its biologically inactive isomer (12). High doses of inositol, 10 mg per rat ICV, were required, but the brain contains high concentrations of inositol (17). We estimated that if 10 mg of inositol ICV were necessary for a 1 g rat brain, over 10 g/kg inositol might be required to achieve central effects after peripheral administration. Using the sensitive model of Li-pilocarpine seizures, preliminary results of peripheral inositol administration were encouraging (3). We hereby report more extensive results.

METHOD

Biochemical Study

Forty male Sprague-Dawley rats (weight 394 ± 26 g) were randomly divided into four groups. Two groups received 3 mEq LiCl/kg IP and the two other groups — 3 mEq NaCl/kg (both 0.15 M). After 24 h animals received IP 10 g/kg glucose or 10 g/kg inositol (10% solution in 0.15 M saline). Each rat was killed by decapitation 6 h later. Inositol levels were

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determined in replicate samples from left cerebral cortex after storage for 10–30 days at −70°C.

Analysis of Inositol Levels

Free myo-inositol was analyzed in brains of rats as trimethylsilyl (TMS) derivates by gas-liquid chromatography, as previously described by Allison et al. (2) with minor modifications as follows: samples of tissue (approximately 50 mg) were dissected from the cerebral cortex, weighed, extracted in 0.5 ml of boiling water for 5 min, the denatured tissue spun down and 250 μl supernatant lyophilized (3 h, Speed Vac SC 110); silylation of the dried sample was carried out with 200 μl of mixture of pyridine : bis(trimethylsilyl) trifluoroacetamide : chlorotrimethylsilan 10 : 2 : 1 (v/v/v) for 24 h at room temperature. Aliquots (2 μl) were chromatographed on a 6-ft column packed with 3% SE-30 on 80/100 mesh gas chrome Q (Supelco), using a Carlo Erba SCU 600 gas chromatograph with a hydrogen flame ionization detector. The oven temperature was isothermal at 200°C and the carrier gas was nitrogen with a flow of 120 ml/min. The trimethylsilyl derivates of myo-inositol had a retention time of 11 min. Under these conditions, quantitation was performed with the use of TMS derivates of standard myo-inositol under the same conditions. Standard curves were run daily, and linearity was verified at the beginning and periodically during the processing of the samples.

Behavioral Study

Sprague-Dawley male rats (weight 380 g) received 3 mEq LiCl/kg IP (0.15 M) and were randomly divided into two groups. After 24 h the animals were separated into individual boxes and received 20 mg/kg pilocarpine (SC). Prior to the pilocarpine injection (Experiment A) one group received 5 g inositol/kg IP (1.4 M in 0.15 M saline) and the second group—5 g glucose/kg IP (1.4 M in 0.15 M saline). After pilocarpine injection animals were placed in clear polycarbonate cages and scored by an observer who was blind to treatment condition. Behavior was rated for signs of seizure according to a modified version of the scale used by Patel et al. (16): 0—no response; 1—gustatory movements and/or fictive scratching; 2—tremor and/or hind limb extension; 3—head bobbing; 4—forelimb clonus; 5—rearing, clonus and falling. Each animal was observed for 15 s once every 5–10 min for 100 min. The latency to attain forelimb clonus (a score of 4) was recorded for each rat. The experiment was then repeated in a new group of rats using 10 g/kg inositol or glucose (Experiment B); and again using 12 g/kg inositol or glucose (Experiment C).

Animals receiving 12 g/kg inositol IP in 10% solution were lethargic for 1–2 h after injection but were normally active by 6 h, before pilocarpine was injected.

TABLE 1

LEVELS OF INOSITOL (mmol/kg wet weight; MEAN ± SD) IN RAT CORTEX FOLLOWING INTRAPERITONEAL INJECTIONS OF 10 g/kg GLUCOSE OR INOSITOL

Intraperitoneal Injection	Pretreatment	
	NaCl (3 mEq/kg)	LiCl (3 mEq/kg)
Glucose	4.35 ± 1.47 (n = 10)	4.20 ± 1.40 (n = 10)
Inositol	5.89 ± 1.78 (n = 10)	5.36 ± 1.63 (n = 10)

Two-way ANOVA showed a significant effect of inositol (f = 7.27, p = 0.01) but no effect of Li (f = 0.47) and no interaction between inositol and Li treatments (f = 0.14).

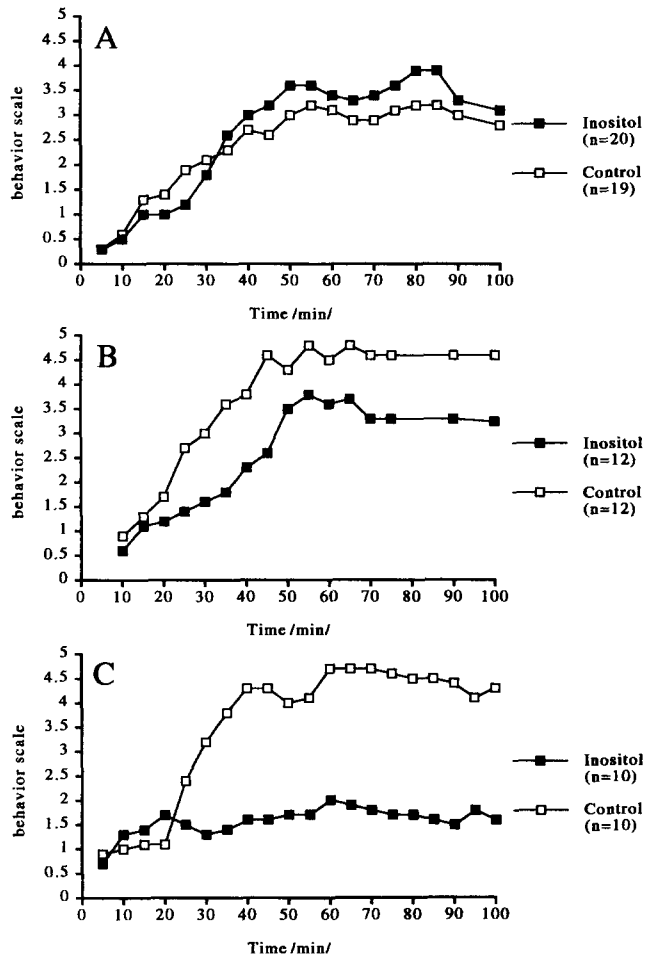

FIG. 1. (A) Li-pilocarpine behavior in rats pretreated with acute low-dose (5 g/kg) inositol IP or control. (B) Li-pilocarpine behavior in rats pretreated with 10 g/kg inositol IP or control; (C) Li-pilocarpine behavior in rats pretreated with 12 g/kg inositol IP or control.

RESULTS

The acute biochemical results are presented in Table 1. Two-way ANOVA showed a significant effect of inositol (F = 7.27, p = 0.01) but no effect of Li (F = 0.47) and no interaction between inositol and Li treatments (F = 0.14). Li 3 mEq/kg reduces brain inositol levels by about 10%, as reported by Sherman et al. (18), but this reduction was not statistically significant. Inositol 10 g/kg raised brain inositol levels by about 25% in Li-treated rats and in Na-treated rats.

Figure 1 illustrates the behavioral effects of acute inositol. Low-dose 5 g/kg inositol (A) has no effect on Li-pilocarpine seizures. MANOVA shows a significant effect of time only (F = 31.82, p < 0.001), but no effect of inositol and no interac-

tion between drug and time. There was no difference in latency to clonus between rats that received inositol or glucose (43.4 ± 12.3 min vs. 44.7 ± 16 min, respectively). Ten grams per kilogram inositol (B) has an intermediate effect: MANOVA shows a significant effect of inositol ($F = 13.5$, $p < 0.001$) and of time ($F = 24.1$, $p < 0.001$) and a significant interaction between inositol and time ($F = 1.9$, $p = 0.036$). At every time point after 25 min the inositol group had a lithium-pilocarpine behavioral score significantly lower than that of the group of rats pretreated with glucose ($p < 0.01$, Newman-Keuls post hoc test). Latency to clonus was significantly ($p < 0.02$) prolonged by 10 g/kg inositol (46 ± 8 min) as compared to glucose (33 ± 9 min). But there was no difference in number of rats attaining forelimb clonus—9 of 12 inositol pretreated rats and 12 of 12 glucose pretreated rats ($\chi^2 = 3.43$, $p = 0.064$). Using 12 g/kg inositol (C), MANOVA shows a significant effect of inositol ($F = 16.9$, $p < 0.001$) and of time ($F = 13.0$, $p < 0.001$) and a significant interaction between inositol and time ($F = 7.8$, $p < 0.001$). At every time point after 25 min the inositol group had a lithium-pilocarpine behavioral score significantly lower than that of the group of rats pretreated with glucose ($p < 0.001$, Newman-Keuls posthoc test). The number of rats attaining forelimb clonus (score 4 and 5) was 9 of 10 glucose pretreated rats, but only 4 of 10 inositol pretreated rats ($\chi^2 = 5.6$, $p = 0.02$).

DISCUSSION

Inositol 5 g/kg was unable to reverse Li-pilocarpine seizures, whereas 12 g/kg was highly effective. This is consistent with ICV studies, where 10 mg inositol ICV raises brain inositol by almost 100% (12) and prevents Li-pilocarpine seizures, whereas 5 mg ICV inositol is barely effective behaviorally (6). Thus, the steep dose-response curve for IP inositol effects on Li-pilocarpine seizures is consistent with a similar steep dose-response curve and requirement for high inositol doses in ICV studies.

The present results confirm preliminary behavioral effects of IP inositol reported previously (3). Although the doses required to achieve behavioral effects in this study are high, it should be noted that inositol is a natural food constituent that is sweet-tasting and metabolized rapidly with about 1/3 the caloric value of glucose. Given a dose ratio of 10:1 for rat: human doses, a 70 kg person might require for reversal of central Li effects about 100 g per day. Thus doses of 12.5 g/kg in the rat are possibly equivalent to human doses of about three inositol-sweetened ice-cream cones daily. This might be tolerated as well as a 100 g glucose load in a glucose tolerance test. Pharmacokinetic studies of inositol administration to rat and human are required. Clements et al. (7) administered 3 g of inositol orally to human volunteers and reported that blood inositol levels tripled. Levine et al. (15) found that 12 g daily of inositol significantly increased human CSF inositol levels by 70% in eight patients. The present results support the possibility that high doses of oral inositol may have psychoactive effects in humans (14) and that oral inositol in lower doses may have peripheral effects in humans but not psychoactive effects (5).

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REFERENCES

1. Allison, J. H.; Stewart, M. A. Reduced brain inositol in lithium-treated rats. Nature New Biol. 233:267-268; 1971.
2. Allison, J. H.; Blisner, M. E.; Holland, W. M.; Hipps, P. P.; Sherman, W. B. Increased brain myo-inositol 1-phosphate in lithium-treated rats. Biochem. Biophys. Res. Commun. 71:664-670; 1976.
3. Belmaker, R. H.; Revazov, A.; Bersudsky, Y.; Kofman, O. Inositol passes the blood-brain barrier sufficiently to reverse lithium effects on behavior in rats. Clin. Neuropharmacol. 15:606A-607A; 1992.
4. Berridge, M. J.; Downes, C. P.; Hanley, M. R. Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. Biochem. J. 206:587-595; 1982.
5. Bersudsky, Y.; Vinnitsky, I.; Grisaru, N.; Yaroslavsky, U.; Gheorghiu, S.; Ivgi, D.; Kofman, O. and Belmaker, R. H. The effect of inositol on lithium-induced polyuria-polydipsia in rats and humans. Hum. Psychopharmacol. 7:403-407; 1992.
6. Bersudsky, Y.; Vinnitsky, I.; Grisaru, N.; Kofman, O.; Belmaker, R. H. Dose-response and time-curve of inositol prevention of Li-pilocarpine seizures. Eur. Neuropsychopharmacol. 3: 428-429; 1993.
7. Clements, R. S., Jr.; Reynertson, R. Myoinositol metabolism in diabetes mellitus. Effect of insulin treatment. Diabetes 26:215-221; 1977.
8. Drummond, A. H. Lithium and inositol lipid-linked signalling mechanisms. Trends Pharmacol. Sci. Rev. 8:129-133; 1987.
9. Honchar, M. P.; Olney, J. W.; Sherman, W. R. Systemic cholinergic agents induce seizures and brain damage in lithium-treated rats. Science 220:323-325; 1983.
10. Kofman, O.; Belmaker, R. H. Intracerebroventricular myo-inositol antagonizes lithium-induced suppression of rearing behavior in rats. Brain Res. 534:345-347; 1990.
11. Kofman, O.; Belmaker, R. H.; Grisaru, N.; Alpert, C.; Fuchs, I.; Katz, V.; Rigler, O. Myo-inositol attenuates two specific behavioral effects of acute lithium in rats. Psychopharmacol. Bull. 27:185-190; 1991.
12. Kofman, O.; Sherman, W. R.; Katz, V.; Belmaker, R. H. Restoration of brain myo-inositol levels in rats increases latency to lithium-pilocarpine seizures. Psychopharmacology (Berlin) 110: 229-234; 1993.
13. Kofman, O.; Bersudsky, Y.; Vinnitsky, I.; Alpert, C.; Belmaker, R. H. The effect of peripheral inositol injection on rat motor activity models of depression. Israel J. Med. Sci. 29: 580-586; 1993.
14. Levine, J.; Gonzalves, M.; Barbur, I.; Stir, S.; Elizur, A.; Kofman, O.; Belmaker, R. H. Inositol 6 g daily may be effective in depression but not in schizophrenia. Hum. Psychopharmacol. 8: 49-53; 1993.
15. Levine, J.; Rapaport, A.; Lev, L.; Bersudsky, Y.; Kofman, O.; Belmaker, R.; Shapiro, J.; Agam, G. Inositol treatment raises human CSF inositol levels. Brain Res. 627:168-170; 1993.
16. Patel, S.; Meldrum, B. S.; Fine, A. Susceptibility to pilocarpine-induced seizures in rats increases with age. Behav. Brain. Res. 31: 165-167; 1988.
17. Sherman, W. R. Lithium and the phosphoinositide signalling system. In: Birch, N. J., ed. Lithium and the cell. London: Academic Press Ltd.; 1991:121-157.
18. Sherman, W. R.; Munsell, L. Y.; Gish, B. G.; Honchar, M. P. Effects of systemically administered lithium on phosphoinositide metabolism in rat brain, kidney and testis. J. Neurochem. 44: 798-807.
19. Spector, R.; Lorenzo, A. V. Myo-inositol transport in the central nervous system. Am. J. Physiol. 228:1510-1518; 1975.