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Augmentation of lithium's behavioral effect by inositol uptake inhibitors

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Summary. Lithium inhibits the enzyme inositol monophosphatase and thus obstructs the enzymatic degradation of inositol triphosphate (IP_3) to inositol in the phosphate-phosphoinositide (PIP) cycle. This inhibition may result in reduced availability of the second messengers IP_3 and DAG that are derivates of the PIP cycle, and this action is currently a leading hypothesis regarding lithium's therapeutic and prophylactic effect in affective disorders. Inositol is also available to the cell by uptake from the intercellular matrix, and therefore it is possible that compounds that block the uptake may have lithium-like effects. To test this hypothesis, the present study evaluates the effects of two inositol uptake inhibitors, the carbohydrate L-fucose and the cyclodepsipeptide nordidemnin, in a behavioral model of pilocarpine-induced seizures known to be enhanced by lithium. We tested the possibility that L-fucose produces lithium-like effects, or that L-fucose or nordidemnin augment lithium's behavioral effects.

Results indicate that acute ICV treatment with L-fucose did not by itself have a lithium-like effect in the behavioral model, but significantly augmented lithium's effect when combined with lithium treatment. Nordidemnin treatment showed similar effects.

The results suggest that when inositol monophosphatase is inhibited by lithium, further restriction of cellular inositol availability may result in an augmentation of lithium's behavioral effects. It is possible that such manipulations may be applicable in the treatment of patients with affective disorders, especially patients who are poor responders to lithium monotherapy.

Keywords: Bipolar disorder, inositol, uptake, lithium, fucose, nordidemnin

Introduction

The carbohydrate inositol is an isomer of glucose that plays a crucial role in the inositol phosphate-phosphoinositide (PIP) cycle (Batty and Downes,

1995; Berridge et al., 1982; Drummond, 1987; Regan, 1990; Fisher et al., 1992). The PIP cycle is the source of two important second messengers: IP_3 that causes an increase of free cytosolic Ca^{2+}, and DAG that activates protein kinase C. These second messengers play a role in cholinergic (muscarinic receptors), noradrenergic (α1 receptors) serotoninergic (5-HT_{2A} and 5-HT_{2C} receptors) and certain other transmitter systems (Drummond, 1987).

Within the PIP cycle, the enzymatic degradation of IP_3 to inositol is obstructed by lithium which uncompetitively inhibits the enzyme inositol monophosphatase (Hallcher and Sherman, 1980), resulting in a reduced availability of inositol substrate for the cycle, and in an impaired ability of cells to react to transmitters acting through the above second messengers. The reduction in cellular inositol levels and ensuing inhibition of the PIP cycle is currently the leading hypothesis regarding lithium's therapeutic and prophylactic effect in affective disorders (Berridge et al., 1982, 1989).

In addition to degradation through the PIP cycle, inositol's cellular levels also derive from uptake from the intercellular matrix. Inositol is present in normal diet which provides approximately 1 g per day, and is also formed from glucose in a number of organs including testis, liver, kidney, and brain (Aukema and Holub, 1994). In man, inositol is present in plasma in concentrations of 30–60 μM (Aukema and Holub, 1994; Agam et al., 1995), and it can be slowly transported across the blood brain barrier (Patishi et al., 1996b; Spector and Lorenzo, 1975). A number of in vitro experiments demonstrated avid uptake of inositol from extracellular medium into cells (Batty et al., 1993; Glanville et al., 1989; Wiesinger, 1991).

Based on the inositol depletion hypothesis of lithium's action, it may be possible that other compounds that reduce inositol levels may have lithium-like effects and thus possible clinical relevance. Since cellular inositol levels are also affected by uptake, it is conceivable that inhibition of the uptake mechanism may have lithium-like effects (Hertz et al., 1997; Wolfson et al., 1998).

To test this hypothesis, the present study evaluates the effects of two inositol uptake inhibitors: The carbohydrate L-fucose and the cyclodepsipeptide nordidemnin. L-fucose was demonstrated to inhibit inositol uptake to cultured cells (Johnson et al., 1993; Stafani et al., 1992; Yorek et al., 1992). Furthermore, Yorek et al. (1993) demonstrated that chronic treatment with oral L-fucose resulted in reduced inositol levels in nerve cells, as well as in changes in activity of these nerves. These changes are reversible by exogenous inositol supplementation (Yorek et al., 1993). Nordidemnin was studied in cultured cells only, and was demonstrated to be a potent inhibitor of inositol uptake at relatively minute doses (Dominice et al., 1994). To evaluate behavioral consequences of treatment with inositol uptake inhibitors, the present study utilized a model of pilocarpine induced seizures. Whereas lithium by itself does not have proconvulsant effects in rats (Ormandy et al., 1991), pretreatment with lithium results in limbic seizures after subconvulsant doses of the cholinergic agonist pilocarpine (Honchar et al., 1983). This effect was found to be reversed by exogenous myo-inositol that reduced the number of rats reaching seizures, and increased the latency to seize after lithium pilocarpine treatment (Bersudsky et al., 1994; Kofman et al., 1993), but was not

affected by the inactive isomer L-chiro-inositol. Since the appearance of seizures after subconvulsant doses of pilocarpine appears to be highly sensitive to cellular inositol level, the model seems to be appropriate for the purpose of the present study.

Because of unknown penetration of these compounds from blood stream into the brain, we chose to administer them directly into the brain via intracerebroventricular cannulae.

Methods

Subjects

Sprague-Dawley male rats (N = 41 for experiment 1; N = 24 for experiment 2; and N = 32 for experiment 3), weighing 200–250 gm at the beginning of experiment served as subjects. Rats were implanted with an intracerebroventricular cannulae to permit ICV injections. After surgery, rats were singly housed in a temperature controlled (22°C) colony room, with a 12 hr. light/ dark cycle, and with free access to food and water. To reduce stress associated with the experimental procedure, rats were handled by the experimenter for a week prior to the beginning of experiment, 2 minutes every day. All experimental and test procedures were performed during the later part of the light phase.

Drugs

Lithium Chloride was diluted in distilled water to give an injection volume of 10 ml/kg, and injected IP (3 meq/kg). L-fucose and D-fucose (SIGMA, Israel), 20 mg/40 μl were diluted in artificial CSF without glucose and injected ICV. Nordidemnin (experiment 3, from P. Jouin, for synthesis details see Jouin et al., 1991) was first diluted in DMSO and then further diluted 1:100 in artificial CSF, and injected (1 μM/10 μl) ICV. Pilocarpine (30 mg/kg) was diluted to 30 mg/ml in distilled water and injected SC. Artificial CSF is composed of 140 mM NaCl; 3 mM KCl; 1.5 mM $CaCl_2$; 1.0 mM $MgCl_2$; 1.2 mM NaH_2PO_4; and 0.27 mM Na_2HPO_4.

Surgical procedure

Rats were anesthetized with xylazine (Vitamed, Il; 12 mg/kg) and ketalar (Park Davis, Gwent, U.K.; 75 mg/kg) and implanted with guide cannulae (Plastics One, Products, Roanoke, VA) into the lateral ventricle (randomly to right or left ventricle) using standard stereotaxic procedures. Coordinates for the cannula were 0.8 mm posterior to bregma, 1.4 mm lateral to midline, and 5 mm below the skull. At least 7 days recovery were allowed following surgery.

Experimental procedure

Each experiment had 3 phases: (1) lithium or control pretreatment, (2) ICV drug treatment, and (3) pilocarpine treatment. The specific treatment for each group in each experiment is detailed in Table 1. Immediately after the last phase (pilocarpine treatment), rats were observed and scored for the appearance and timing of clonic seizures. After the appearance of seizures, or after 90 minutes in case no seizures were demonstrated, rats were anesthetized with nembutal (Ceva, Paris, France: 30 mg/kg) and then decapitated. Brains were extracted and location of cannulae was examined.

Data analysis

For experiment 1, since none of the rats from the saline-fucose group reached clonus, and all the rats of the other two groups (pretreated with lithium) demonstrated seizures, only

Table 1. Drug and treatment regimes

	Experiment 1	Experiment 2	Experiment 3
Pretreatment – 22–24 hours before start of experiment	Group Saline-Fucose: saline IP Groups Li-fucose and Li-CSF: 3 meq/kg lithium IP	Both groups: 3 meq/kg lithium IP	Both groups: 3 meq/kg lithium IP
ICV drug treatment	3 hours before start of experiment	3 hours before start of experiment	At 36, 24, and 2 hours before start of experiment
	Group Li-CSF: 40 μl artificial CSF ICV	Group L-fucose: 20 mg L-fucose ICV	Group Nordid: 1 μM in 10 μl nordidemnin ICV
	Groups Saline-focuse and Li-fucose: 20 mg L-fucose in 40 μl artificial CSF ICV	Group D-fucose: 20 mg D-fucose ICV *both drugs in 40 μl artificial CSF	Group CSF: 10 μl CSF
At start of the experiment	All groups: 30 mg/kg pilocarpine SC	Both groups: 30 mg/kg pilocarpine SC	Both groups: 30 mg/kg pilocarpine SC

data for the Li-fucose and Li-CSF groups was analyzed. A Student's t-test was utilized for the analysis of the time of appearance of clonic seizures. In Experiment 2, many of the rats did not reach clonus and a X^2 analysis was utilized for the rate of appearance of clonus. For experiment 3, a student's t-test was used to compare the time of appearance of clonic seizures.

Results

Experiment 1

A single ICV injection of 20 mg L-fucose, administered 3 hours before pilocarpine treatment, did not induce seizures in rats that were not pretreated with lithium, and none of the 14 rats of the group reached clonus (data not shown). However, rats pretreated with lithium and treated with ICV L-fucose before pilocarpine, reached clonus faster than the control (CSF) group (Fig. 1; t-test: $t = 2.192$, $p = 0.038$).

Experiment 2

L-fucose potentiated lithium-pilocarpine seizures when compared with D-fucose (a relatively inactive isomer of L-fucose) used to control for osmotic or other nonspecific effects. Since only a relatively low number of the control

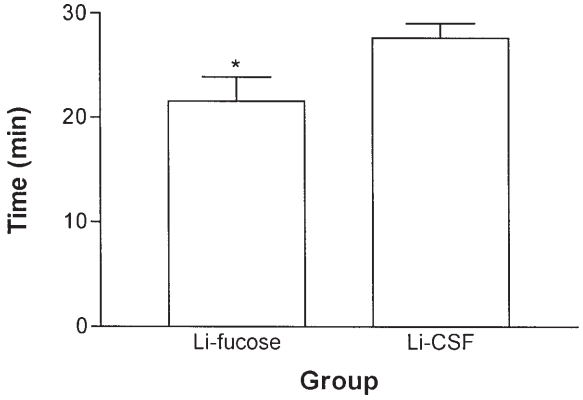

Fig. 1. Time after pilocarpine injection to exhibit clonic seizures in rats pretreated with lithium and treated with 20 mg ICV L-fucose (N = 14) or CSF control solution (N = 13), mean + SE. Groups are significantly different (2 tail t-test: t = 2.192, *p = 0.038)

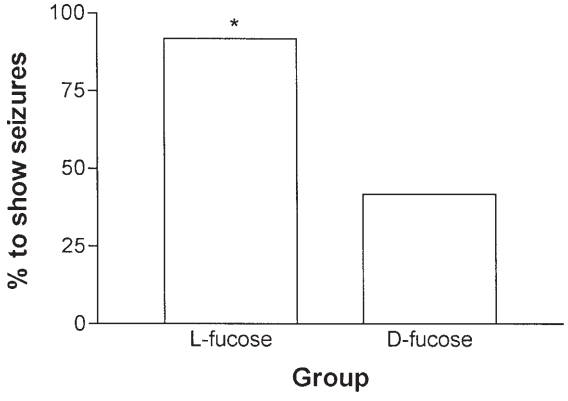

Fig. 2. The percentage of rats (N = 12 for each group) that exhibited clonic seizures after lithium pretreatment, 20 mg ICV L-fucose or D-fucose, and pilocarpine, mean + SE. Groups are significantly different (X^2 test: $X^2(1) = 6.75$, $p < 0.01$)

group rats demonstrated clonus, it was not possible to compare the mean time to reach seizures. Instead, Fig. 2 shows the percentage of animals that reached clonus after L-fucose compared to D-fucose (X^2 test: $X^2(1) = 6.75$, $p < 0.01$). All animals received lithium pretreatment and pilocarpine treatment as detailed in the methods section.

Experiment 3

As shown in Fig. 3, rats pretreated with lithium and treated with nordidemnin, had a shorter latency to pilocarpine induced seizures than the control group (one tailed t-test: t = 2.009, p = 0.027).

An attempt was also made to test the effects of a third inositol uptake inhibitor, scyllo-inositol (Johnson et al., 1993); however, it was not possible to

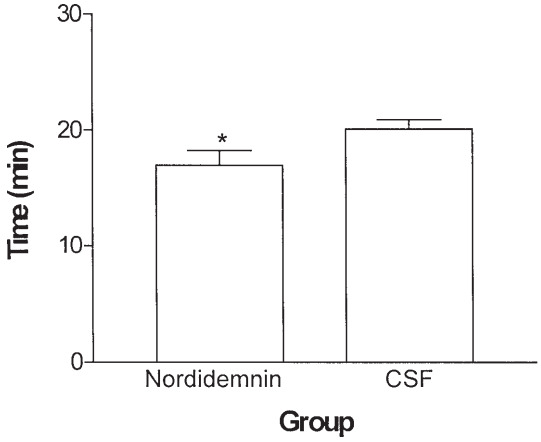

Fig. 3. Time after pilocarpine injection to exhibit clonic seizures in rats (N = 16 for each group) pretreated with lithium and treated repeatedly with ICV nordidemnin or CSF control solution, mean + SE. Groups are significantly different (one tail t-test: t = 2.009, p = 0.027)

dissolve the compound to any higher concentration than 1 mg/40 μl, and there was no effect at this low dose (data not shown).

Discussion

The results of the present study indicate that ICV treatment with inositol uptake inhibitors may augment lithium's action in potentiating pilocarpine induced seizures, but seem not to be able to replace lithium in this model. The results support the possibility that inhibition of inositol uptake into the cell may produce behavioral effects in the same direction as those produced by lithium. Although treatment with the inositol uptake inhibitor L-fucose was not able to replace lithium in a model of pilocarpine induced seizures, the same treatment significantly augmented lithium's action. Similar results were demonstrated after treatment with the inositol uptake inhibitor nordidemnin.

Most of the cell's inositol supply is derived from turnover in the PIP cycle, and the contribution of uptake to cellular inositol levels may be less than 5% (Glanville et al., 1989). However, in the presence of lithium that inhibits both inositol's turnover in the PIP cycle, and its de-novo synthesis in the brain (Sherman et al., 1985), the relatively small amounts of inositol that enter the cell via the uptake mechanism may come to play a more significant role. After stimulation with pilocarpine and the resultant depletion of inositol by PIP cycle overactivity (Sherman et al., 1985), the uptake of exogenous ICV inositol has been shown to delay and even prevent lithium-pilocarpine seizures (Kofman et al., 1993). The present results suggest that even in the absence of exogenous inositol, CSF may contain some inositol whose uptake delays the appearance of lithium-pilocarpine seizures. By inhibiting inositol uptake and blocking this reserve, lithium-pilocarpine seizures are accelerated as intracellular inositol reaches inadequate levels faster.

The dose of L-fucose used in the study was 20 mg. Considering that the volume of rat brain is approximately 1 ml, the concentration reached in the brain can be assumed to be at the range of 10 times the concentration that had an inositol uptake inhibition effect in cultured cells (Stefani et al., 1992). Similarly, the amount of nordidemnin used was also aimed to reach a brain concentration of approximately 10 times the concentration found to inhibit inositol uptake in cultured cells (Dominice et al., 1994).

A dose response curve for L-fucose and nordidemnin and the effect of chronic treatment should be studied in the future. However, the positive nature of the results suggest that inositol uptake inhibitors can augment lithium's action. The clinical relevance of the present study may be more important in regard to L-fucose than to nordidemnin, a cyclodepsipeptide that degrades quickly in the human body. In contrast, L-fucose is a sugar that could enter the brain after peripheral administration. The penetration of L-fucose through the blood drain barrier, and into the brain remains to be studies. If indeed peripherally administered compound could augment lithium's effects, the clinical significance of inositol uptake inhibitors could be considerable, as a possible additive to lithium's treatment in non-responders or partial-responders patients who are afflicted with bipolar disorder.

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