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Lithium-induced Increase of Synaptosomal Uptake of Norepinephrine in Rat Brain

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Lithium remains the most widely used therapeutic agent for bipolar affective disorder, particularly mania. Although many investigators have studied the effects of lithium on abnormalities in monoamine neurotransmitter as a pathophysiological basis of affective disorder, the action mechanism of lithium ion remains still unknown. To explore the action mechanism of lithium in the brain, we examined the effects of lithium on the extrasynaptosomal concentrations of catecholamines and their metabolites. Synaptosomes were prepared from the rat forebrains and assays of catecholamines and metabolites were made using HPLC with an electrochemical detector. Lithium of 1mM decreased the extrasynaptosomal concentrations of NE from the control group of 3.07 ± 1.19 to the treated group of 0.00 ± 0.00 (ng/ml of synaptosomal suspension) but not that of DHPG. It can be suggested that lithium increases synaptosomal uptake of NE. Increased intraneuronal uptake of NE would decrease neurotransmission and extraneuronal metabolism of NE. Because increased brain NE metabolism and neurotransmission have been suggested as important components in the pathophysiology of bipolar affective disorder, especially mania, lithium-induced increase of intraneuronal NE uptake can be suspected as an action mechanism of therapeutic effect of lithium in manic patient, possibly in bipolar affective disorder.

Key Words: Lithium, Norepinephrine, HPLC, Synaptosome

INTRODUCTION

Mood (affective) disorders are currently categorized as either bipolar (manic-depressive) or unipolar (depressive). Bipolar disorder is further characterized according to whether the current or most recent episode is manic, depressive, or mixed (concurrent or rapidly alternating manic and depressive features).

Many investigators have suggested that abnormalities in monoamine neurotransmitters including norepinephrine (NE), dopamine (DA), and serotonin (5-HT) were involved in the pathophysiology of bipolar affective disorder. NE neurotransmission might be decreased in depression (Mass, 1978; Schildkraut et al, 1984) and possibly increased in mania (Annitto & Shopsin, 1979; Post, 1980). DA neurotransmission might be decreased in depression and increased in mania with psychotic features (Willner, 1983). 5-HT neurotransmission was decreased in depression and mania (Van Praag, 1977; Price et al, 1990).

Lithium carbonate is the treatment of choice of affective disorders, particularly mania, and widely used for the prevention of recurrent attacks of bipolar manic-depressive illness. It is also sometimes used as an augmenting agent in patients who have not had a response to antidepressant drugs alone (Bowden et al, 1994; Price & Heninger, 1994; Manji et al, 1995b).

The biological basis for the clinical efficacy of lithium is unknown, although some effects on the regulation of synaptic neurotransmission are suspected. There are many studies about the effect of lithium on abnormalities in monoamine neurotransmitters as the pathophysiological basis of affective disorder. In aspects of effects of lithium on the synaptosomal release of catecholaminergic neurotransmitters, Baldessarini & Vogt (1988) have demon-

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strated that lithium at concentrations of 1 to 10 mEq/l inhibited the depolarization-provoked and Ca^{2+}-dependent release of norepinephrine and dopamine, but not 5-HT, from nerve terminals (synaptosomes) of animal brain. There are some reports about effects of lithium on the metabolism of catecholamines. Clinically, lithium has been shown to increase or have no effect on 5-HT turnover (Price et al, 1990), to have little effect or decrease NE turnover (Linnoila et al, 1983b), and to have inconsistent effects on or possibly decrease DA turnover (Linnoila et al, 1983a). But, in animal studies about the effect of lithium on the metabolism of brain catecholamine, there are few reports and results are not clear and variable (Schildkraut, 1974).

Isolated nerve terminals (synaptosomes) are widely used to study the secretion of neurotransmitters and the presynaptic stimulus-secretion coupling in the CNS. Because the isolated nerve terminals retain a number of the properties of CNS neurons (Erecinska & Dagani, 1990), a high membrane potential, tight bioenergetic coupling, and the ability to release amino acid neurotransmitters (Nicholls, 1989; Verhage et al, 1989), catecholamines (Drapeau & Blaustein, 1983; Woodward et al, 1988), and neuropeptides (Floor, 1983; Verhage et al, 1991).

From the above historical background of lithium as a therapeutic agent for the affective disorder, we have investigated effects of lithium on extrasynaptosomal concentrations of catecholamines and their metabolites to elucidate the action mechanism of lithium in the brain.

METHODS

Materials

Lithium carbonate, NaH_2PO_4, disodium EDTA, 1-octanesulfonic acid, ethanol, phosphoric acid, methanol, norepinephrine, dihydroxyphenylglycol (DHPG), epinephrine, L-dihydroxyphenylalanine (L-DOPA), dopamine, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxytryptamine (5-HT, serotonin), 5-hydroxyindoleacetic acid (5-HIAA), dihydroxybenzylamine (DHBA) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade.

Preparation of synaptosome

Adult male Sprague-Dawley rats (250~300 g) were used in all the experiments. Synaptosomes were prepared from forebrains by some modifications of the method of Booth & Clark (1978). In brief, rats were killed by decapitation and the forebrains of the animal were rapidly removed. The forebrains were dropped into ice-cold isolation medium (0.32 M-sucrose/1 mM-potassium EDTA/ 10 mM-Tris/HCl, pH 7.4) and chopped into small pieces with scissors. The blood and other debris were washed off the brain tissue by adding more isolation medium and decanting the supernatant from the top of the minced tissue. This washing procedure was repeated. The chopped tissue was then homogenized in a Brinkmann homogenizer (Polytron). This homogenate was diluted to 60 ml with isolation medium and spun at 1,300 *g* for 3 min in a Beckman J2-21 centrifuge at 4°C. The supernatant from this spin was centrifuged at 17,000 *g* for 10 min, producing the crude mitochondrial/synaptosomal pellet. This pellet was resuspended in 3 ml of isolation medium, diluted to 12 ml with 12% Ficoll sucrose medium [12% (w/w) Ficoll, 0.32 M sucrose, 50 μM-potassium EDTA, pH 7.4] and gently homogenized by a Brinkmann homogenizer (Polytron).

The crude mitochondrial suspension was introduced into a centrifuge tube and above this, 3 ml of 7.5% Ficoll/sucrose medium [7.5% (w/w) Ficoll, 0.32 M-sucrose, 50 μM-potassium EDTA, pH 7.4] was carefully layered. Finally, on top of this 3 ml of isolation medium was layered. The tubes were centrifuged at 99,000 *g* for 30 min in a swing-out rotor in a Beckman L8-80 ultracentrifuge.

Myelin and synaptosomes banded at the first and second interphases respectively, with the free mitochondria being pelleted at the bottom. The myelin layer was carefully removed and the synaptosomes were gently sucked off from the interphase. The synaptosomes were diluted to 30 ml with isolation medium and gently homogenized as above in a Brinkmann homogenizer. The synaptosomes were finally diluted to 60 ml and spun at 5,500 *g* for 10 min.

The final synaptosomal pellet from four rats was taken up in isolation medium in a final volume of 30 ml. This synaptosomal suspension contained 0.269 mg protein/ml and was stored at −70°C until use.

Protein determination

Protein content of the synaptosomal suspension was determinated as described by Lowry et al (1951) using bovine serum albumin as a standard.

Assay of catecholamine

Preparation of sample solution for catecholamine assay: The measurement of extrasynaptosomal catecholamine concentration was made according to the method of Garcia et al (1994) with some modifications. One-tenth of a milliliter of the synaptosomal suspension and 0.1 ml of 10 mM lithium carbonate (final concentration of 1 mM at the reaction mixture of 1ml) were added to 0.7 ml of artificial CSF solution (containing in mM 25 Tris-HCl, 120 NaCl, 3 KCl, 2 $MgCl_2$, 2 $CaCl_2$, pH 7.35). The reaction mixture was preincubated for 5 min at 37°C; 0.1 ml of 10 mM ATP (final concentration of 1 mM at the reaction mixture) was added, and the mixture was incubated for 60 min at 37°C. The reaction was stopped by the addition of 1 ml of ice-cold 10% trichloroacetic acid, and the solution was centrifuged at 3,300 *g* for 30 min to remove protein. 1 ml of the protein-free supernatant was aspirated and mixed with 1 ml of DHBA standard solution (200ng of DHBA/ 1 ml). 2 ml of DHBA-containing supernatant was passed through a 0.2 μm membrane filter and used for the sample solution of the lithium-treatment group for the analysis of extrasynaptosomal concentration of catecholamines.

Analysis of extrasynaptosomal catecholamine concentration in the normal control group was measured as described above but, the reaction mixture contained 0.1 ml of synaptosomal suspension, 0.7 ml of artificial CSF solution and 0.1 ml of distilled water instead of lithium carbonate.

Apparatus: A sample solution of 10 μl was injected onto a Waters isocratic HPLC system consisting of a pump (Waters 510) and a Waters 460 electrochemical detector set at 600 mV and 15 nA of sensitivity.

Separation was carried out on a Waters μ-Bondapak C18 column (3.9×300 mm I.D.). The signal from the electrochemical detector was recorded on a Waters 745B integrator.

The composition of the mobile phase per liter was 13.8 g of NaH_2PO_4, 60 mg of Na_2EDTA, 20 mg of 1-octanesulfonic acid and 2 % ethanol. The apparent pH was adjusted to 3.70 with H_3PO_4 before addition of ethanol. The flow-rate was 1.0 ml/min.

Catecholamine standard solution: Catecholamine standard solution contained 0.1 ng/10μl of norepinephrine(NE), dihydroxyphenylglycol (DHPG), epinephrine (EP), L-dihydroxyphenylalanine (L-DOPA), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxytryptamine (5-HT, serotonin), 5-hydroxyindoleacetic acid (5-HIAA), and dihydroxybenzylamine (DHBA) as an internal standard.

Statistical analysis

Statistical analyses were done according to Student's *t*-test. *p* values below 0.05 were regarded as significant.

RESULTS

A chromatogram of catecholamine standards

Fig. 1 shows a chromatographic recording of catecholamine standards. A volume of 10 μl containing 0.1 ng of NE, DHPG, EP, L-DOPA, DA, DOPAC, HVA, 5-HT, 5-HIAA, and DHBA (internal standard) was injected. Retention time of each peaks were as follows (in min); NE (4.32), L-DOPA (5.18), DHPG (5.82), EP (6.99), DHBA (7.82), DA (9.84), DOPAC (11.79), 5-HT (13.76), 5-HIAA (18.32), and HVA (24.40) (Fig. 1).

Chromatographic recordings of catecholamines in the control group and the lithium-treated group

Fig. 2 shows two chromatographic recordings of the extrasynaptosomal catecholamines in the control group (A) and the lithium-treated group (B). In the control group, NE, DHPG, EP, DA, 5-HT, and 5-HIAA were detected. In the lithium-treated group, peaks of DHPG, EP, DA, 5-HT, and 5-HIAA were recorded but NE was not detected. In the both groups, L-DOPA, DOPAC, and HVA were not detected. Although both chromatographic analyses have been interrupted at the retention time of about 30min to

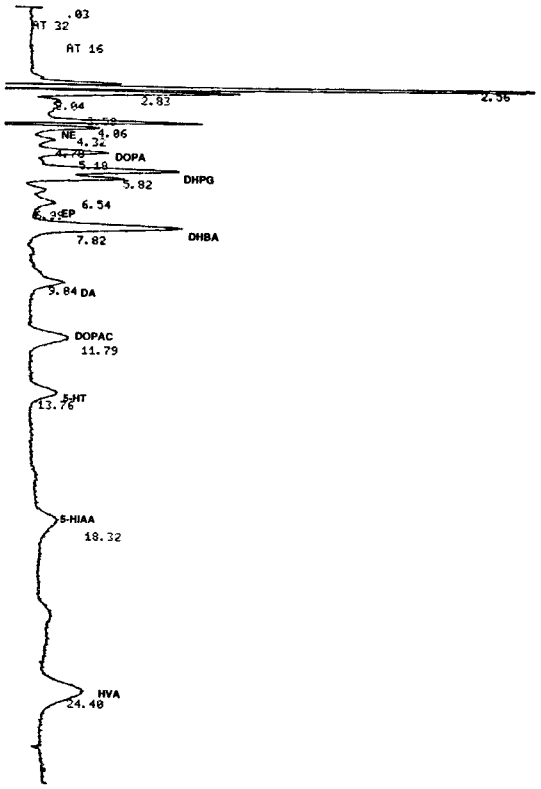

Fig. 1. A chromatographic recording of catecholamine standards. A volume of 10 μl containing 0.1 ng of norepinephrine(NE), L-dihydroxyphenylalanine (DOPA), dihydroxyphenylglycol (DHPG), epinephrine (EP), dihydroxybenzylamine (DHBA), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptamine (5-HT, serotonin), 5-hydroxyindoleacetic acid (5-HIAA), and homovanillic acid (HVA) was injected. Numbers represent the retention times (in min) of each peaks of standards. The chromatographic condition was described in the Materials and Methods.

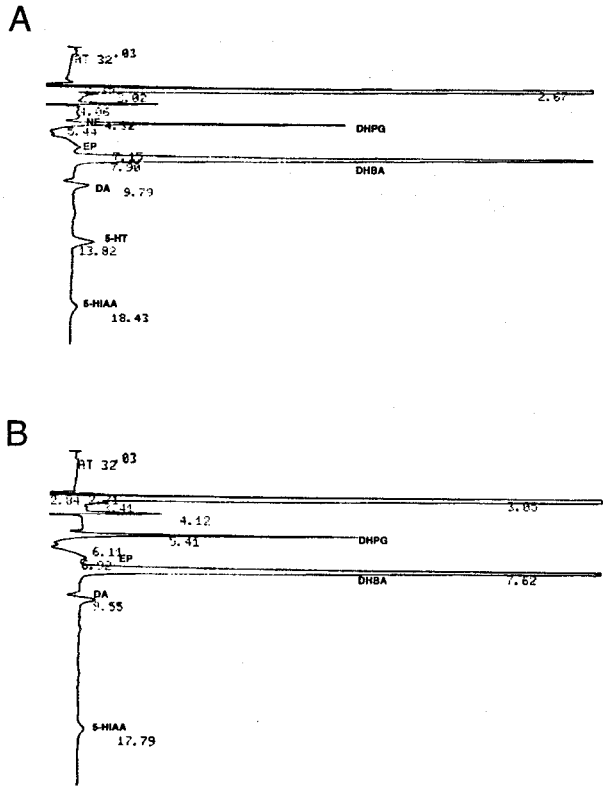

Fig. 2. Chromatographic recordings of the extrasynaptosomal catecholamines in the control group (A) and the lithium-treated group (B). A volume of 10 μl of sample solution containing 1 ng of DHBA as an internal standard was injected. Numbers represent the retention times (in min) of each peaks of standards. The chromatographic condition was described in the Materials and Methods.

record the peak of HVA, each chromatograms on Fig. 2 do not show the full recordings.

Effects of lithium on the extrasynaptosomal concentrations of catecholamines

Concentrations of catecholamines in both the control and the lithium-treated groups were summarized in Table 1. Compared to the control group, extrasynaptosomal amounts of NE, DHPG, EP, and 5-HT were decreased, that of DA was decreased, and that of 5-HIAA was not changed by the treatment of lithium carbonate at the concentration of 1mM. But, the change of NE concentration was only statistically significant ($p<0.05$). The extrasynaptosomal concentration of NE was decreased from the control level of 3.07 ± 1.19 ng/ml to the lithium-treated level of 0.00 ± 0.00 ng/ml (mean $\pm$ S.E. of 6 experiments).

DISCUSSION

Lithium remains the most widely used therapeutic agent for bipolar affective disorder, particularly mania (Bowden et al, 1994; Price & Heninger, 1994; Manji et al, 1995b). Although many investigators have studied the effects of lithium on abnormalities in monoamine neurotransmitter as a pathophysiological basis of affective disorder, the action mechanism of lithium ions remains still unknown. In order to explore the

Table 1. Extrasynaptosomal concentrations of catecholamines in the control group and the 1mM lithium-treated group

catecholamines	control group	lithium-treated group
NE	3.07±1.19	*0.00±0.00
DHPG	2.33±0.51	2.21±0.34
EP	0.85±0.10	0.80±0.06
DA	0.39±0.01	0.45±0.04
5-HT	0.28±0.09	0.07±0.07
5-HIAA	0.11±0.02	0.11±0.04

Each data represent the mean±S.E.M. from 6 experiments.

Catecholamine concentrations were expressed as ng/ml of synaptosomal suspension.

* significantly different from the normal control group ($p<0.05$).

NE, norepinephrine; DHPG, dihydroxyphenylglycol; EP, epinephrine; DA, dopamine; 5-HT, 5-hydroxytryptamine (serotonin); 5-HIAA, 5-hydroxyindoleacetic acid.

mechanism of action of lithium in the brain, we examined effects of lithium on extrasynaptosomal concentrations of catecholamines and their metabolites. Synaptosomes were prepared from the rat forebrains. Because the optimum therapeutic concentration range of lithium in plasma is 0.6~1.5 mM (or 0.75~1.25 mEq/l), all experiments were performed at the concentration of lithium carbonate of 1 mM.

The result of this study was summarized in Table 1. Lithium of 1mM decreased the extrasynaptosomal concentrations of NE from the control group of 3.07 ±1.19 to the treated group of 0.00±0.00 (ng/ml of synaptosomal suspension) and slightly decreased that of DHPG from 2.33±0.51 to 2.21±0.34. The ratio of DHPG/NE was increased in the lithium-treated group. This result indicates that lithium at the optimum therapeutic concentration of 1 mM does not decrease the release of NE from synaptosome but increases the synaptosomal uptake of NE and the rate of NE turnover as estimated by the ratio of DHPG to NE. But it is not reasonable to discuss about the ratio of NE turnover because the NE content of lithium-treated group is not detectable. This result is partly consistent with the report that lithium at the concentration of 1 to 10 mEq per liter inhibited the release of NE from the nerve terminals (Baldessarini & Vogt, 1988).

Increased brain NE turnover and metabolism (Young et al, 1994), increased urinary NE excretion (Koslow et al, 1983; Swann et al, 1983; Swann et al, 1990), and increased NE neurotransmission (Mass, 1978; Annitto & Shopsin, 1979; Post, 1980; Schildkraut et al, 1984) have been suggested as important components in the pathophysiology of bipolar affective disorder, especially mania.

Eisenhofer et al (1992) indicated that the increased intraneuronal uptake of NE will decrease neurotransmission, extraneuronal uptake, and extraneuronal metabolism of NE. So, lithium-induced increase of intraneuronal NE uptake can be suspected as an action mechanism of therapeutic effect of lithium in manic patient, possibly in bipolar affective disorder.

As shown in Table 1, the extrasynaptosomal concentration of DA was increased by lithium of 1mM from the control group of 0.39±0.01 to the treated group of 0.45±0.04 (ng/ml), but this change was statistically not significant ($p>0.05$). HVA as a main metabolite of DA was not detected in this study. Although statistically not significant, it can be suggested from the result of lithium-induced increase of extrasynaptosomal DA concentration that synaptosomal uptake of DA would be decreased or synaptosomal release of DA would be increased. But, this result is not consistent with other reports that lithium has moderately attenuated DA function in animal (Price & Heninger, 1994) and has inhibited the release of DA from nerve terminals (Baldessarini & Vogt, 1988). The cause of this inconsistency may be due to the differences of chromatographic conditions.

The concentration of 5-HT was decreased by the treatment of 1mM lithium from the control group of 0.28±0.09 to the treated group of 0.07±0.07 (ng/ml), but that of 5-HIAA as a main metabolite was not changed (Table 1). Statistically not significant ($p>$ 0.05), the ratio of 5-HIAA/5-HT was increased from 0.39 to 1.57. From this result, two hypotheses can be suggested. First, lithium increases the uptake of 5-HT. Second, lithium decreases the release of 5-HT. But, both two hypotheses are not consistent with other reports that lithium increases the synthesis and turnover of 5-HT in presynaptic neurons (Price et al, 1990) and that lithium enhances the release of 5-HT in the brain (Grahame-Smith & Green, 1974; De-

Montigny et al, 1981; Treiser et al, 1981). The cause of this inconsistency may be due to the differences of chromatographic conditions.

From this study it can be concluded that lithium of 1mM decreased extrasynaptosomal concentration of NE but do not alter that of DHPG. So, we can suggest that lithium increases synaptosomal uptake of NE instead of release of NE from synaptosome. The increased intraneuronal uptake of NE would decrease neurotransmission, extraneuronal uptake, and extraneuronal metabolism of NE. Because the increased brain NE turnover and neurotransmission have been suggested as important components in the pathophysiology of bipolar affective disorder, especially mania, lithium-induced increase of intraneuronal NE uptake can be suspected as a action mechanism of therapeutic effect of lithium in manic patient, possibly in bipolar affective disorder.

On the other hand, recent studies have focused on the molecular mechanisms of lithium as a mood-stabilizing agent. There has been a growing appreciations that neurotransmitter function might be altered indirectly through alterations in intracellular signaling, and mood-stabilizing agents such as lithium might be effective not because they are catecholaminergic or cholinergic agents per se but because they alter the postsynaptic signal generated in response to multiple, endogenous neurotransmitters (Manji et al, 1995a). Recent evidence has also demonstrated significant effects of lithium on the regulation of gene expression in the central nervous system, effects that may play a major role in the long-term stabilization of mood (Manji et al, 1995b).

According to the above recent studies, results of the present study will be further evaluated to elucidate the molecular mechanism of the relation between the lithium-induced modulation of catecholaminergic neurotransmission and the therapeutic effect of lithium.

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