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Effect of co-administration of lithium and reboxetine on extracellular monoamine concentrations in rats

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Abstract

We investigated the effect of reboxetine, a selective noradrenaline reuptake inhibitor, 7 days after treatment with subchronic lithium on extracellular noradrenaline, dopamine and serotonin (5-HT) concentrations in the medial prefrontal cortex. Acute treatment with reboxetine significantly increased extracellular concentrations of noradrenaline and dopamine, but did not alter 5-HT concentrations. Subchronic lithium increased basal levels of extracellular 5-HT, but not noradrenaline or dopamine. Co-administration of reboxetine and lithium treatment increased the extracellular concentrations of noradrenaline, dopamine and 5-HT, though reboxetine alone increased the extracellular levels of noradrenaline and dopamine only. Thus, combined lithium and reboxetine produces an additive effect neurochemically rather than their interaction.

Keywords: Serotonin; Noradrenaline; Dopamine; Reboxetine; Lithium carbonate; In vivo microdialysis

1. Introduction

Clinically, randomized controlled trials have shown lithium augmentation of the effect of antidepressants, which inhibit the reuptake of noradrenaline, or serotonin (5-hydroxytryptamine, 5-HT), or both (Thase and Rush, 1995). Subchronic or chronic lithium increases the antidepressant effect of the noradrenaline reuptake inhibitor desipramine in non-responders to it (Joffe et al., 1993). Previous studies suggests that the mode of action of the lithium increase of the antidepressant effect of selective 5-HT reuptake inhibitors is partly mediated by increased 5-HT neurotransmission by lithium (Wegener et al., 2000; Muraki et al., 2001). However, neurochemical experiments of combined treatment with lithium and selective noradrenaline reuptake inhibitors have not been reported.

Reboxetine is a highly selective noradrenaline reuptake inhibitor with no marked affinity for muscarinic, histamine, or dopamine receptors or adrenoceptors, and has an advantage in a use for basic research over desipramine because of its high selectivity (Wong et al., 2000). Recent clinical evidence has shown that reboxetine is effective in the treatment of major depression (Ban et al., 1998). The antidepressant effects of reboxetine have been investigated by behavioral and neurochemical studies. Microdialysis studies have shown that acute and chronic treatments with reboxetine increase extracellular noradrenaline and dopamine concentrations in the medial prefrontal cortex, but do not alter extracellular 5-HT concentrations (Dekeyne et al., 2001; Page and Lucki, 2002).

To clarify the mode of action of lithium augmentation of the antidepressant effect of noradrenaline reuptake inhibitors, this study examined if subchronic lithium treatment affects acute reboxetine action on extracellular dopamine, noradrenaline and 5-HT concentrations in the medial prefrontal cortex of rats by using an in vivo microdialysis method. Previous microdialysis studies have reported the effect of lithium on extracellular dopamine and 5-HT concentrations in the brain (Baptista et al., 1993; Pei et al., 1995; Wegener et al., 2000; Muraki et al., 2001), we believe the effects of lithium on extracellular noradrenaline concentrations have not been studied.

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2. Material and methods

2.1. Animals

Male Sprague–Dawley rats obtained from the Shizuoka Laboratory Animal Center (Shizuoka, Japan), weighing 180–280 g, were housed in groups of four and were maintained in a 12-h light–dark cycle (light phase: 06:30–18:30), temperature-controlled environment (22 ± 1 °C) with free access to food and water. The rats were maintained on a diet of standard laboratory rat chow alone. Fourteen days after acclimatization, rats were fed rat chow containing 0.2% Li_2CO_3 for 7 days, and control rats were fed rat chow without Li_2CO_3. Both lithium-treated rats and the control rats were given 10 mM NaCl instead of tap water to prevent lithium-induced hyponatremia.

2.2. Experimental design

Rats received standard rat chow alone, or rat chow containing 0.2% of Li_2CO_3 for 7 days. On day 6, rats were implanted stereotaxically with guide cannulae leading to the surface of the medial prefrontal cortex, and dialysis probes were inserted into the guide cannulae. After surgery, rats were given standard laboratory rat chow or rat chow containing 0.2% of Li_2CO_3 and 10 mM NaCl. Twenty hours after surgery, using artificial cerebrospinal fluid started perfusion. Two hundred minutes after the start of the collection of dialysate samples, rats received a single injection of reboxetine at a dose of 1 or 10 mg/kg.

Reboxetine methansulphonate (Pharmacia & Upjohn, Milano, Italy) was dissolved in 0.9% saline as 1 ml/kg and was injected intraperitoneally (i.p.).

2.3. Microdialysis procedures

2.3.1. Surgery and perfusion

The rats were implanted stereotaxically under pentobarbital anesthesia (30 mg/kg i.p.) with AG-4 guide cannulae (Eicom, Kyoto, Japan) leading to the surface of the medial prefrontal cortex at the ffsollowing coordinates relative to the bregma: A +3.2 mm, ML +0.8 mm, DV +1.0 mm. Dialysis probes with an outer diameter of 0.22 mm (A-I-4-03; Eicom) were then inserted into the guide cannulae so that 3.0 mm of the probe was exposed to the tissue of the medial prefrontal cortex. Rats were housed individually after these operations.

The experiments were done with in freely moving rats. Twenty hours after surgery, perfusion was started by using artificial cerebrospinal fluid (145 mM NaCl, 3.0 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgCl_2$) at a flow rate of 1 μl/min. Following initial perfusion for 2 h, dialysate samples were collected in sample vials containing 50 μl of 0.05 M acetic acid every 40 min for 480 min. Thirty-microliter dialysate samples were injected into an high-performance liquid chromatography (HPLC) system to measure the extracellular levels of noradrenaline, and 20-μl dialysate samples were injected into an HPLC system to measure the extracellular levels of dopamine and 5-HT.

These procedures were approved by the Hokkaido University Graduate School of Medicine Animal Care and Use Committee and complied with the Guide for the Care and Use of Laboratory Animals, Hokkaido University Graduate School of Medicine.

2.3.2. Analytical procedures of noradrenaline

The HPLC system consisted of an EP-300 liquid chromatograph pump (Eicom), a DG-300 degasser (Eicom), a reversed phase ODS column, Eicompak CA-5ODS 150 2.1 mm (Eicom), an ECD-300 electrochemical detector (Eicom) and a PowerChrom (AD Instruments, Sydney, Australia). The mobile phase was 0.1 M phosphate buffer (pH 6.0) containing 5% (v/v) methanol, 50 mg/l Na_2 EDTA and 500 mg/l L-octanesulfonate. The separations were at 25 °C with a flow rate of 0.23 ml/min. The electrochemical detector was set at an oxidation potential of 550 mV. A solution of standard noradrenaline was injected every working day and the amount of extracellular noradrenaline in the rats was compared with the peak height of the standard noradrenaline.

2.3.3. Analytical procedures of dopamine and 5-HT

We used the same chromatograph pump, degasser, electrochemical detector and a PowerChrom as used for the noradrenaline analysis. The HPLC system included a reversed phase ODS column, Eicompak PP-ODS 30 4.6 mm (Eicom). The mobile phase was 0.1 M phosphate buffer (pH 6.0) containing 1% (v/v) methanol, 50 mg/l Na_2 EDTA and 500 mg/l sodium L-decanesulfonate. The separations were at 25 °C with a flow rate of 0.5 ml/min. The electrochemical detector was set at an oxidation potential of 400 mV. A solution of standard dopamine and 5-HT was injected every working day, and the amount of extracellular dopamine and 5-HT was compared with the peak height of the standard dopamine and 5-HT.

2.4. Statistical analysis

All data are the mean ± S.E.M. of individual values of rats from each group. The noradrenaline, dopamine and 5-

Table 1
Effect of subchronic lithium treatment (0.2% Li_2CO_3 for 7 days orally) on basal levels of extracellular noradrenaline, dopamine and 5-HT in the medial prefrontal cortex

	Normal diet controls	0.2% Li_2CO_3
Noradrenaline	0.770 ± 0.074	0.656 ± 0.062
Dopamine	0.865 ± 0.090	0.856 ± 0.072
5-HT	0.793 ± 0.122	1.721 ± 0.331[a]

Values represent the mean ± S.E.M. (pg/40 min fraction). 5-HT, $N=20$ (normal diet), $N=19$ (lithium diet); dopamine, $N=22$ (Normal diet), $N=23$ (lithium diet); noradrenaline, $N=22$ (normal diet), $N=19$ (lithium diet).

[a] $P<0.05$ vs. the normal diet group.

HT amounts in the dialysate samples were expressed as absolute values (pg/fraction). Repeated measures analysis of variance (ANOVA) for absolute values was used to examine the interaction between lithium treatment and time factors (0–240 min). The average of absolute values of extracellular noradrenaline, dopamine and 5-HT in five consecutive samples (−160 to 0 min) before injection of reboxetine was calculated as basal levels. Differences in absolute values at each time point between the normal diet group and 0.2% Li_2CO_3 group (reboxetine 1 mg/kg i.p. and 10 mg/kg i.p.) were analyzed by using an unpaired *t*-test (two-tailed). The time points of each group were compared by using a paired *t*-test. The statistical significance was $P < 0.05$.

3. Results

3.1. Effect of subchronic 0.2% Li_2CO_3 treatment on basal levels of extracellular noradrenaline, dopamine and 5-HT in the medial prefrontal cortex

The basal levels of extracellular noradrenaline or dopamine between the 0.2% Li_2CO_3 group and normal diet

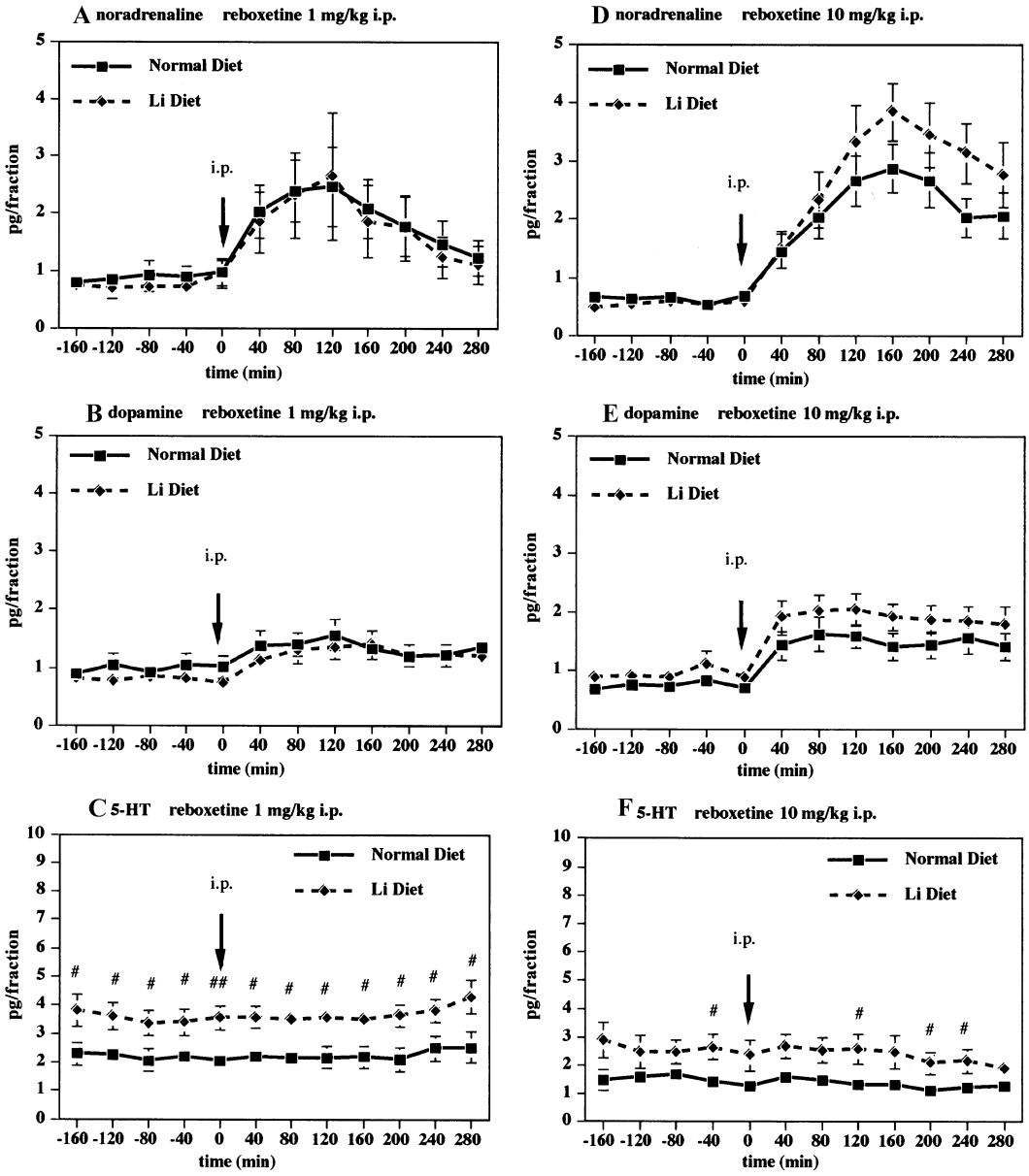

Fig. 1. Effect of acute reboxetine (1 or 10 mg/kg i.p.) on extracellular concentrations of noradrenaline, dopamine and 5-HT in the medial prefrontal cortex after subchronic lithium treatment (0.2% Li_2CO_3 for 7 days orally). Values represent the mean ± S.E.M. (pg/40 min fraction). (A) $N = 12$ (normal diet), $N = 10$ (lithium diet); (B) $N = 12$; (C) $N = 10$ (normal diet), $N = 9$ (lithium diet); (D) $N = 10$ (normal diet), $N = 9$ (lithium diet); (E) $N = 11$ (normal diet), $N = 10$ (lithium diet); (F) $N = 9$ (normal diet), $N = 10$ (lithium diet). $^{\#\#}P < 0.01$, $^{\#}P < 0.05$ vs. the normal diet group.

group showed no statistical differences. Basal extracellular 5-HT levels of the 0.2% Li_2CO_3 group were significantly higher than those of the normal diet group (unpaired *t*-test, $P<0.05$) (Table 1).

3.2. Effect of acute reboxetine on extracellular noradrenaline, dopamine and 5-HT concentrations in the medial prefrontal cortex after subchronic 0.2% Li_2CO_3 treatment (Fig. 1)

Acute reboxetine (1 and 10 mg/kg i.p.) increased extracellular noradrenaline concentrations, but subchronic 0.2% Li_2CO_3 treatment did not affect the effects of reboxetine (Fig. 1A,D). Two-way ANOVA with repeated measures (0–240 min) indicated a significant main effect of time [1 mg/kg, $F(6,120)=12.717$, $P<0.0001$; 10 mg/kg, $F(6,102)=23.556$, $P<0.0001$]. The 0.2% Li_2CO_3 treatment showed no significant main effect, and the interaction between 0.2% Li_2CO_3 treatment and time was not significant. Acute reboxetine (1 and 10 mg/kg) with normal diet increased extracellular noradrenaline significantly compared with basal levels of extracellular noradrenaline (paired *t*-test, 40 to 280 min, $P<0.01$). Acute reboxetine (1 and 10 mg/kg) with lithium diet also increased extracellular noradrenaline significantly compared with basal levels of extracellular noradrenaline (paired *t*-test, 1 mg/kg, 40 to 240 min, $P<0.01$, 280 min, $P<0.05$; 10 mg/kg, 40 to 280 min, $P<0.01$).

Acute reboxetine (1 and 10 mg/kg i.p.) increased extracellular dopamine concentrations, but subchronic 0.2% Li_2CO_3 treatment did not affect the effects of reboxetine (Fig. 1B,E). Two-way ANOVA with repeated measures (0–240 min) indicated only a significant main effect of time [1 mg/kg, $F(6,132)=8.573$, $P<0.001$; 10 mg/kg, $F(6,114)=19.516$, $P<0.0001$]. No significant main effect of 0.2% Li_2CO_3 treatment or no interaction between 0.2% Li_2CO_3 treatment and time was shown. Acute reboxetine (1 and 10 mg/kg) with normal diet increased extracellular dopamine significantly compared with basal levels of extracellular dopamine (paired *t*-test, 1 mg/kg, 40 to 160, 240, 280 min, $P<0.01$, 200 min, $P<0.05$; 10 mg/kg, 40 to 280 min, $P<0.01$). Acute reboxetine (1 and 10 mg/kg) with lithium diet also increased extracellular dopamine significantly compared with basal levels of extracellular dopamine (paired *t*-test, 40 to 280 min, $P<0.01$).

Acute reboxetine (1 and 10 mg/kg i.p.) did not change extracellular 5-HT concentrations, but subchronic 0.2% Li_2CO_3 treatment increased both basal levels and levels after reboxetine administration of extracellular 5-HT. Two-way ANOVA with repeated measures (0–240 min) indicated significant main effects of 0.2% Li_2CO_3 treatment [1 mg/kg, $F(1,17)=14.220$, $P<0.005$; 10 m/kg, $F(1,17)=6.983$, $P<0.05$]. No main effect of time and no interaction between 0.2% Li_2CO_3 treatment and time was shown, except significant main effect of time for 10 mg/kg [$F(6,102)=2.640$, $P<0.05$]. The lithium diet group showed significantly higher concentrations of extracellular 5-HT compared with the normal diet group (unpaired *t*-test, 1 mg/kg, −160 to −40, 40 to 280 min, $P<0.05$, 0 min, $P<0.01$; 10 mg/kg, −40, 120, 200, 240 min, $P<0.05$).

4. Discussion

In this study, subchronic 0.2% Li_2CO_3 treatment for 7 days increased the basal levels of extracellular 5-HT in the medial prefrontal cortex. Our results agree with recent in vivo microdialysis studies, in which the basal levels of extracellular 5-HT after subchronic lithium treatment increased in the medial prefrontal cortex and ventral hippocampus of the unanesthetized rats (Wegener et al., 2000; Muraki et al., 2001). Increased 5-HT release in lithium-treated rats might be explained by previous biochemical studies on the effects of lithium administration in vivo that showed increased synthesis of 5-HT in the whole brain (Sheard and Aghajanian, 1970), and an increased 5-HT turnover in various brain regions of rats (Eroglu and Hizal, 1987). In our previous study, plasma lithium levels were 0.71 ± 0.05 mEq/l after 0.2% Li_2CO_3 treatment for 7 days (Muraki et al., 1999). Therefore, subchronic lithium treatment facilitates 5-HT release at therapeutic plasma levels of lithium.

In this study, acute treatment with the selective noradrenaline reuptake inhibitor reboxetine significantly increased extracellular noradrenaline and dopamine concentrations of the medial prefrontal cortex, agreeing with a previous study (Page and Lucki, 2002). In contrast to the effect on 5-HT, subchronic 0.2% Li_2CO_3 treatment did not affect basal levels of extracellular noradrenaline or dopamine in the medial prefrontal cortex, and it did not influence the effects of reboxetine on extracellular dopamine or noradrenaline concentrations. These results indicate that subchronic lithium treatment does not affect the facilitation by reboxetine of noradrenergic and dopaminergic neurotransmission in the medial prefrontal cortex.

Acute reboxetine did not affect extracellular 5-HT concentrations in the medial prefrontal cortex, agreeing with a previous study (Page and Lucki, 2002). In this study, coadministration of reboxetine and lithium induced significantly higher concentrations of extracellular 5-HT in the medial prefrontal cortex than reboxetine alone. Thus, subchronic lithium adds the effects of the increase in extracellular 5-HT concentrations to the effects of the increase in extracellular noradrenaline and dopamine concentrations induced by acute reboxetine. Although this study has the limitation that it examined the effect of acute antidepressant administration to normal rats, indicating difficulty of concluding the relevance to the clinical action of antidepressants, no valid animal model of major depression has been reported until now. This study suggests that lithium augmentation of noradrenaline reuptake inhibitors is mediated by the addition of increases in the basal levels of extracellular 5-HT by lithium administration.

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