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Effect of milnacipran on extracellular monoamine concentrations in the medial prefrontal cortex of rats pre-treated with lithium

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Abstract

Antidepressants are effective in most patients with depression, but sometimes have sub-optimal effects. Thus, there is a need to use more powerful antidepressants when dealing with treatment-resistant cases. Lithium carbonate is widely used for this purpose. We investigated the acute effects of milnacipran, a serotonin-noradrenaline reuptake inhibitor, on extracellular serotonin, dopamine and noradrenaline concentrations, in the rat medial prefrontal cortex. The effects of milnacipran were examined in rats following 7 days of treatment with lithium, and in untreated controls. The lithium group had significantly greater basal levels of extracellular serotonin than the control group. Milnacipran (3 mg/kg) combined with lithium treatment caused a greater increase in extracellular noradrenaline and dopamine levels than milnacipran alone. Milnacipran (3 and 30 mg/kg) combined with lithium treatment also caused a greater increase in extracellular serotonin levels than milnacipran alone. Thus, lithium might augment the antidepressant effects of serotonin-noradrenaline reuptake inhibitors by augmenting serotonin release.
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Keywords: Lithium carbonate; Milnacipran; In vivo microdialysis; Noradrenaline; Dopamine; Serotonin

1. Introduction

Major depressive disorder is a chronic disorder that impairs the social functioning of patients. Acute antidepressant treatment is often required to treat major depressive disorder, as well as long-term maintenance therapy in order to prevent recurrence (Thase, 1999). These patients require antidepressants that are both effective and well-tolerated. Serotonin-noradrenaline reuptake inhibitors are new to clinical practice. The antidepressant effects of milnacipran, a serotonin-noradrenaline reuptake inhibitor, are similar to those of tricyclic antidepressants (Kasper et al., 1996; Van Amerongen et al., 2002; Bisserbe, 2002), but serotonin-noradrenaline reuptake inhibitors are better tolerated (Kasper et al., 1996; Leinonen et al., 1997; Puech et al., 1997; Van Amerongen et al., 2002; Bisserbe, 2002). In double-blind studies, serotonin-noradrenaline reuptake inhibitors demonstrate superior efficacy to selective serotonin reuptake inhibitors in severe cases of depression and are as well tolerated (Rudolph and Feiger, 1999; Clerc and the Milnacipran/Fluvoxamine Study Group, 2001). The results of a meta-analysis further support the superior efficacy of serotonin-noradrenaline reuptake inhibitors over selective serotonin reuptake inhibitors (Lopez-Ibor et al., 1996). Switching from a selective serotonin reuptake inhibitor to a serotonin-noradrenaline reuptake inhibitor is an effective and rational strategy by which to treat selective serotonin reuptake inhibitor non-responders with major depressive disorder (De Montigny et al., 1999). However, despite improvements in the treatment of major depressive disorder following the introduction of serotonin-noradrenaline reuptake inhibitors into clinical practice, treatment-resistant unipolar depression remains a major problem.

To alleviate the symptoms of depression, co-administration of lithium is widely used because of evidence of its effectiveness (Thase and Rush, 1995; Bauer and Döpfmer, 1999). Randomized controlled studies demonstrate success

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of co-administration of lithium with tricyclic antidepressants and selective serotonin reuptake inhibitors (Bauer and Döpfmer, 1999), however, the success of co-administration of lithium with serotonin-noradrenaline reuptake inhibitors remains to be seen. At this point, only open-label trials are available to examine the effect of lithium on serotonin-noradrenaline reuptake inhibitor activity. These trials indicate that co-administration of lithium improves depressive symptoms in non-responders to the serotonin-noradrenaline reuptake inhibitors, venlafaxine and milnacipran (Hoencamp et al., 2000; Kobayashi et al., 2004). Thus, lithium may augment the antidepressant effects of serotonin-noradrenaline reuptake inhibitors, thereby increasing their effectiveness in serotonin-noradrenaline reuptake inhibitor-resistant patients.

Several lines of evidence suggest that, in part, lithium augments the antidepressant effects of other agents by increasing serotonin neurotransmission. In vivo microdialysis experiments show increased extracellular serotonin in the medial prefrontal cortex and hippocampus following daily lithium administration (Wegener et al., 2000; Muraki et al., 2001; Kitaichi et al., 2004). In addition, acute administration of citalopram, a selective serotonin reuptake inhibitor, following a period of lithium administration, results in significantly greater extracellular serotonin concentrations than citalopram alone (Wegener et al., 2000; Muraki et al., 2001). Lithium has pre-synaptic and post-synaptic effects on neurotransmission within serotonergic pathways in the central nervous system (Odagaki et al., 1992). Electrophysiological studies also show increased serotonin neurotransmission due to the pre-synaptic actions of lithium on serotonin nerve terminals (Blier and de Montigny, 1985).

In order to confirm the ability of lithium to augment the activity of serotonin-noradrenaline reuptake inhibitors, an analysis of neurotransmitter levels in the brain is required. In this study, we examined whether treatment with lithium might enhance serotonin-noradrenaline reuptake inhibitor (milnacipran)-induced increases in extracellular serotonin levels in the rat medial prefrontal cortex, in the same way that it does with selective serotonin reuptake inhibitors (Wegener et al., 2000; Muraki et al., 2001), using an in vivo microdialysis method. Extracellular concentrations of noradrenaline and dopamine were examined in the medial prefrontal cortex since the antidepressant effects of milnacipran are known to be mediated by increased catecholamine levels in the rat brain (Moret and Briley, 1997; Mochizuki et al., 2002).

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats from the Shizuoka Laboratory Animal Center (Shizuoka, Japan), weighing 180–280 g, were housed in groups of four and exposed to a 12-h light–dark cycle (light phase: 06:30–18:30) in a temperature-controlled environment (22±1 °C) with free access to food and water. The rats were fed standard laboratory rat chow. Fourteen days after acclimatization, rats in the experimental group were fed rat chow containing 0.2% Li_2CO_3 for 7 days, while control rats were fed rat chow without Li_2CO_3. Both the lithium-treated and control rats were given 10 mM NaCl instead of tap water in order to prevent lithium-induced hyponatremia (Thomsen and Olesen, 1974).

2.2. Experimental design

Rats received standard rat chow alone, or rat chow containing 0.2% Li_2CO_3 for 7 days. On day 6, dialysis probes were inserted through guide cannulae onto the surface of the medial prefrontal cortex using stereotactic guidance. After surgery, rats were given standard laboratory rat chow or rat chow containing 0.2% Li_2CO_3 and 10 mM NaCl. Twenty hours after surgery, the medial prefrontal cortex was perfused with artificial cerebrospinal fluid. Two hundred minutes after the first dialysate samples were collected, rats received a single intraperitoneal injection of milnacipran (Asahi Kasei, Japan) dissolved in distilled water at 1 ml/kg. In our previous study, acute administration of milnacipran (only 30 mg/kg) decreased freezing behavior in the conditioned fear stress model, but milnacipran at doses of 3 or 10 mg/kg failed (Hashimoto et al., 1995). Furthermore, acute milnacipran (30 mg/kg) reduced the immobility time in the forced swimming test, but 10 mg/kg of milnacipran failed (Mochizuki et al., 2002). Thus, 30 mg/kg of milnacipran is functionally effective for these behaviors in rats, but both 3 and 10 mg/kg of milnacipran are ineffective. Therefore, doses of 3 or 30 mg/kg were chosen.

2.3. Microdialysis procedures

2.3.1. Surgery and perfusion

We performed the same in vivo microdialysis procedures as described in a previous paper (Kitaichi et al., 2004). Stereotaxic implantation of AG-4 guide cannulae (Eicom Co., Kyoto, Japan) was performed under pentobarbital anesthesia (30 mg/kg intraperitoneal) leading to the surface of the medial prefrontal cortex. Cannulae were inserted at the following 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 each probe was in contact with tissue of the medial prefrontal cortex. Rats were housed individually after these procedures.

The experiments were done with freely moving rats. Twenty hours after surgery, the medial prefrontal cortex was perfused with artificial cerebrospinal fluid (145 mM NaCl, 3.0 mM KCl, 1.3 mM $CaCl_2$, and 1.0 mM $MgCl_2$) at a flow rate of 1 μl/min. After an initial perfusion period of two hours, dialysate samples were collected every 40 min into vials containing 50 μl of 0.05 M acetic acid over a period of 480 min. Samples (30 μl) of dialysate were injected into a high-performance liquid chromatography system in order to measure the extracellular levels of noradrenaline, and 20 μl samples of dialysate were injected into the high-performance liquid chromatography system in order to measure the extracellular levels of serotonin and dopamine.

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. Analysis of serotonin and dopamine levels

The high-performance liquid chromatography system consisted of an EP-300 liquid chromatograph pump (Eicom, Kyoto, Japan), a DG-300 degasser (Eicom), a reversed phase ODS column, an Eicompak PP-ODS 30 4.6 mm device (Eicom), an ECD-300 electrochemical detector (Eicom), and a PowerChrom device (AD Instruments Pty. Ltd., Sydney, Australia). 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 1-decanesulfonate. Separation was performed at 25 °C using a flow rate of 0.5 ml/min. The electrochemical detector was set at an oxidation potential of 400 mV. Standard solutions of serotonin and dopamine were injected every working day, and the peak extracellular serotonin and dopamine levels were compared with the peak concentrations of the standard serotonin and dopamine solutions.

2.3.3. Analysis of noradrenaline levels

We used the same chromatograph pump, degasser, electrochemical detector and PowerChrom device as described above for analysis of serotonin and dopamine levels. In addition, a reversed phase ODS column, and an Eicompak CA-5ODS 150 2.1 mm device (Eicom) were used. 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. Separation was performed at 25 °C using a flow rate of 0.23 ml/min. The electrochemical detector was set at an oxidation potential of 550 mV. A standard solution of noradrenaline was injected every working day and the peak extracellular noradrenaline concentration of each sample was compared with the peak height of the standard noradrenaline solution.

2.4. Statistical analysis

All data are expressed as the mean±S.E.M. of individual values from rats of each group. Dialysate noradrenaline, dopamine and serotonin concentrations are expressed as absolute values (pg/fraction). Repeated measures analysis of variance (ANOVA) for absolute values was used to examine the effect of milnacipran administration over time in the lithium and control groups (0–240 min). Average absolute values of extracellular serotonin, dopamine and noradrenaline were determined in five consecutive samples (−160 min to 0 min) taken prior to injection of milnacipran in order to determine basal levels. Differences in absolute values of neurotransmitters between the normal diet group and the 0.2% Li_2CO_3 group following intraperitoneal administration of milnacipran (3 mg/kg and 30 mg/kg) were analyzed by using an unpaired t-test (two-tailed). In addition, following the methods of Arborelius et al. (1996) the overall effect of acute milnacipran administration were calculated as the average difference from average basal level over 40–160 min in lithium-treated and control rats given 3 mg/kg milnacipran and 40–240 min in lithium-treated and control rats given 30 mg/kg milnacipran. The overall effects were analyzed by using unpaired *t*-test. Values less than $P<0.05$ were considered statistically significant.

3. Results

3.1. Effect of treatment with 0.2% Li_2CO_3 for 7 days on basal levels of extracellular serotonin, dopamine and noradrenaline in the medial prefrontal cortex

Significantly greater basal levels of extracellular serotonin were observed in the 0.2% Li_2CO_3 group, compared with the normal diet group (unpaired *t*-test, $P<0.01$). This result was reproducible (Muraki et al., 2001; Kitaichi et al., 2004). Significantly greater basal levels of extracellular dopamine were observed in the 0.2% Li_2CO_3 group, compared with the normal diet group in rats scheduled to receive low-dose and high-dose milnacipran. However, this effect was unclear, because the reproducibility was not confirmed in two series of experiments in rats scheduled to receive low-dose and high-dose milnacipran in this study. Statistically significant differences were not observed between basal levels of extracellular noradrenaline in the 0.2% Li_2CO_3 and normal diet groups. We pooled the data obtained from rats scheduled to receive low-dose and high-dose milnacipran (Table 1).

3.2. Acute effects of intraperitoneal milnacipran administration (3 and 30 mg/kg) on extracellular serotonin, dopamine and noradrenaline concentrations in the rat medial prefrontal cortex following treatment with 0.2% Li_2CO_3 for 7 days

Acute milnacipran (3 and 30 mg/kg intraperitoneal) increased extracellular serotonin concentrations (Fig. 1A,D). Two-way ANOVA with repeated measures (0–240 min) indicated significant main effects of 0.2% Li_2CO_3 treatment [3 mg/kg, $F(1,17)=6.124$, $P=0.0242$; 30 mg/kg, $F(1,13)=4.819$, $P=0.0469$] and time [3 mg/kg, $F(6,108)=21.064$, $P<0.001$; 30 mg/kg, $F(6,72)=17.127$, $P<0.0001$], and a significant interaction between 0.2% Li_2CO_3 treatment and time [30 mg/kg, $F(6,72)=2.261$, $P=0.0460$]. In the 3 mg/kg milnacipran treatment, the interaction between 0.2% Li_2CO_3 treatment and time was not significant. The lithium diet group showed significantly higher concentrations of extracellular serotonin compared with the normal diet group (unpaired *t*-test, 3 mg/

Table 1
Effect of lithium treatment (oral administration of 0.2% Li_2CO_3 for 7 days) on basal levels of extracellular serotonin, dopamine and noradrenaline in the medial prefrontal cortex

	Normal diet control group	Lithium carbonate (0.2%) diet group
Serotonin	1.063±0.088	2.200±0.234[a]
Dopamine	0.647±0.041	0.817±0.050[b]
Noradrenaline	1.594±0.122	2.183±0.267

Values represent the mean±S.E.M. (pg/40 min fraction). Data were calculated from Fig. 1. Serotonin, $N=16$ (normal diet), $N=18$ (lithium diet); dopamine, $N=18$; noradrenaline, $N=18$.
[a] $P<0.01$ vs. the control group.
[b] $P<0.05$ vs. the control group.

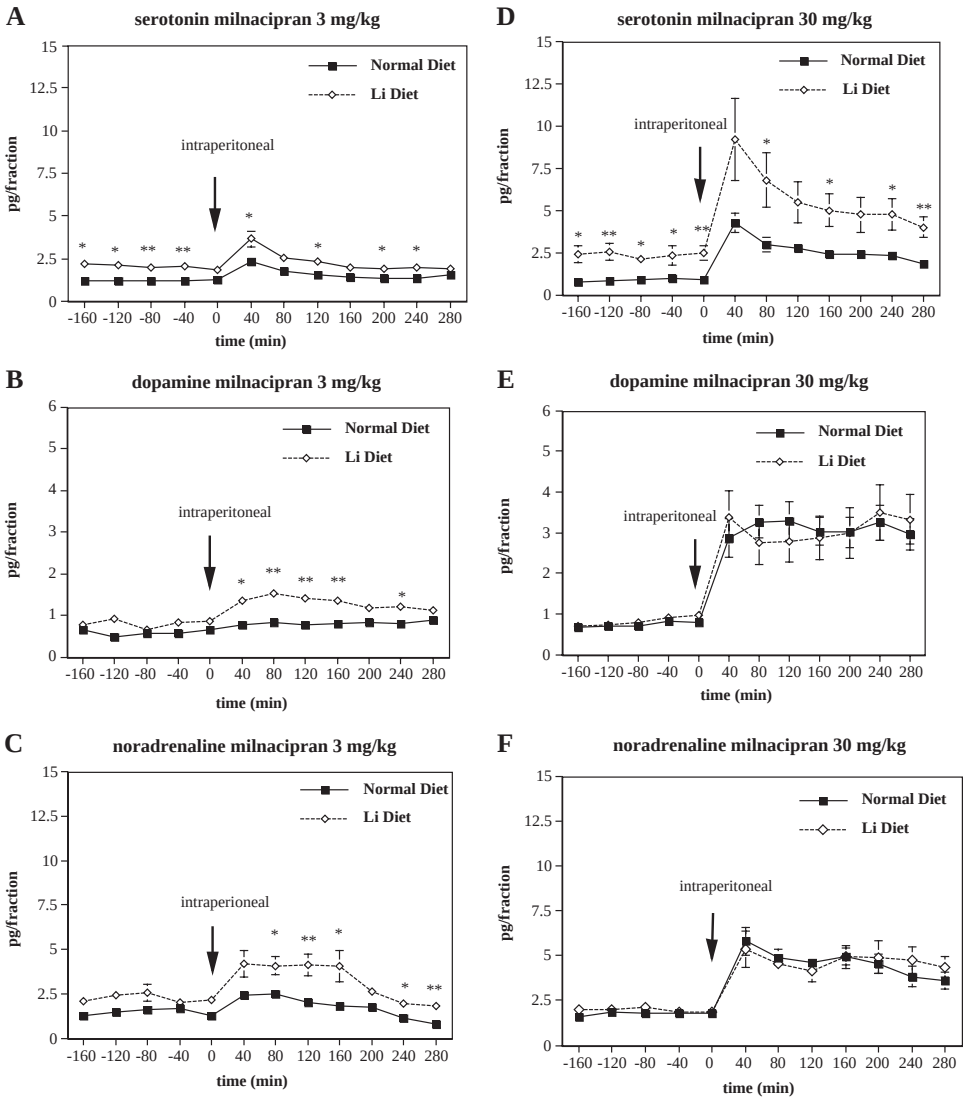

Fig. 1. Effect of acute administration of milnacipran (3 or 30 mg/kg intraperitoneal) on extracellular serotonin, dopamine and noradrenaline concentrations, in the medial prefrontal cortex after 7 days of treatment with lithium (0.2% Li_2CO_3 orally). Values represent the mean ± S.E.M. (pg/40 min fraction). (A) $N=9$ (normal diet), $N=10$ (lithium diet); (B) $N=10$; (C) $N=10$; (D) $N=7$ (normal diet), $N=8$ (lithium diet); (E) $N=8$; (F) $N=8$. $^{**}P<0.01$, $^{*}P<0.05$ vs. the control group.

kg, −80, −40 min, $P<0.01$, −160, −120, 40, 120, 200, 240 min, $P<0.05$; 30 mg/kg, −120, 0, 280 min, $P<0.01$, −160, −80, −40, 80, 160, 240 min, $P<0.05$).

Acute milnacipran (3 and 30 mg/kg intraperitoneal) increased extracellular dopamine concentrations (Fig. 1B,E). Two-way ANOVA with repeated measures (0–240 min) indicated significant main effects of 0.2% Li_2CO_3 treatment [3 mg/kg, $F(1,18)=11.564$, $P=0.032$] and time [3 mg/kg, $F(6,108)=6.519$, $P<0.001$; 30 mg/kg, $F(6,114)=19.516$, $P<0.0001$], and a significant interaction between 0.2% Li_2CO_3 treatment and time [3 mg/kg, $F(6,108)=2.840$, $P=0.0132$]. In the 30 mg/kg milnacipran treatment, 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. The lithium diet group showed significantly higher concentrations of extracellular dopamine compared with the normal diet group (unpaired t-test, 3 mg/kg, 80, 120, 160, $P<0.01$, 40, 240 min, $P<0.05$). In the 30 mg/kg milnacipran treatment, the lithium diet group showed no difference in extracellular dopamine concentrations compared with the normal diet group.

Acute milnacipran (3 and 30 mg/kg intraperitoneal) increased extracellular noradrenaline concentrations (Fig. 1C,F). Two-way

Table 2
Overall difference from basal levels of serotonin, dopamine and noradrenaline outputs (during the 40–160 min interval) in response to milnacipran 3 mg/kg administration

	Normal diet control group	Lithium carbonate (0.2%) diet group
Serotonin	0.544 ± 0.106	0.563 ± 0.112
Dopamine	0.214 ± 0.056	0.607 ± 0.092[a]
Noradrenaline	0.740 ± 0.155	1.863 ± 0.331[a]

Values represent the mean ± S.E.M. (pg). Data were calculated from Fig. 1. Serotonin, $N=9$ (normal diet), $N=10$ (lithium diet); dopamine, $N=10$; noradrenaline, $N=10$.

[a] $P<0.01$ vs. the control group.

Table 3
Overall difference from basal levels of serotonin, dopamine and noradrenaline outputs (during the 40–240 min interval) in response to milnacipran 30 mg/kg administration

	Normal diet control group	Lithium carbonate (0.2%) diet group
Serotonin	1.969±0.244	3.603±0.936
Dopamine	2.386±0.355	2.297±0.577
Noradrenaline	3.007±0.336	2.805±0.484

Values represent the mean±S.E.M. (pg). Data were calculated from Fig. 1. Serotonin, $N=7$ (normal diet), $N=8$ (lithium diet); dopamine, $N=8$; noradrenaline, $N=8$.

ANOVA with repeated measures (0–240 min) indicated significant main effects of 0.2% Li_2CO_3 treatment [3 mg/kg, $F(1,16)=5.866$, $P=0.0262$] and time [3 mg/kg, $F(6,108)=16.046$, $P<0.0001$; 30 mg/kg, $F(6,84)=25.032$, $P<0.0001$], and the significant interaction between 0.2% Li_2CO_3 treatment and time [3 mg/kg, $F(6,108)=2.469$, $P=0.0281$]. In the 30 mg/kg milnacipran treatment, 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. The lithium diet group showed significantly higher concentrations of extracellular noradrenaline compared with the normal diet group (unpaired t-test, 3 mg/kg, 120, 280 min, $P<0.01$, 80, 160, 240 min, $P<0.05$). In the 30 mg/kg milnacipran treatment, the lithium diet group showed no difference in extracellular noradrenaline concentrations compared with the normal diet group.

3.3. Overall effect of 7-day treatment with 0.2% Li_2CO_3 on serotonin, dopamine and noradrenaline release from the medial prefrontal cortex following intraperitoneal administration of milnacipran (3 mg/kg and 30 mg/kg)

Since basal levels of serotonin and dopamine varied considerably, we examined the absolute effect of milnacipran on neurotransmitter release. Compared to the control group, significantly greater increases in extracellular dopamine and noradrenaline were observed 40–160 min following low-dose (3 mg/kg) milnacipran administration in lithium-treated rats, however, this was not the case for serotonin (Table 2). Compared to the control group, significant differences were not observed in extracellular serotonin, dopamine, and noradrenaline levels 40–240 min following high-dose (30 mg/kg) milnacipran administration in lithium-treated rats (Table 3).

4. Discussion

Acute administration of milnacipran (3 and 30 mg/kg) increased extracellular concentrations of serotonin and noradrenaline in the rat medial prefrontal cortex. These results are consistent with those of other studies demonstrating increased extracellular serotonin and noradrenaline concentrations in the medial prefrontal cortex of rats, as well as the hypothalamus of guinea pigs, following administration of milnacipran (Moret and Briley, 1997; Bel and Artigas, 1999; Mochizuki et al., 2002). Similar to duloxetine (Kihara and Ikeda, 1995), acute administration of milnacipran (a serotonin-noradrenaline reuptake inhibitor) also increased extracellular dopamine concentrations. Although milnacipran does not show an affinity for dopamine transporters in rat cortical membranes (Briley et al., 1996; Mochizuki et al., 2002), noradrenaline reuptake inhibition by milnacipran blocks dopamine uptake by noradrenaline transporters, thereby increasing extracellular dopamine in the medial prefrontal cortex (Carboni et al., 1990).

In this study, treatment with 0.2% Li_2CO_3 for 7 days increased basal levels of extracellular serotonin in the medial prefrontal cortex. These results are consistent with a few of recent in vivo microdialysis studies demonstrating increased basal levels of extracellular serotonin in the medial prefrontal cortex and ventral hippocampus of unanaesthetized rats following a period of treatment with Li_2CO_3 (Wegener et al., 2000; Muraki et al., 2001; Kitaichi et al., 2004). Increased serotonin release in Li_2CO_3-treated rats might be related to an increase in serotonin synthesis (Sheard and Aghajanian, 1970; Perez-Cruet et al., 1971; Grahame-Smith and Green, 1974; Berggren, 1985) and increased serotonin turnover (Eroglu and Hizal, 1987; Ghoshdastidar and Poddar, 1990). In a preliminary study, we observed a period of treatment with Li_2CO_3 to elevate K^+-induced increases in extracellular serotonin in the medial prefrontal cortex (Koyama et al., 1991). The results of these studies suggest that Li_2CO_3 treatment facilitates the release of serotonin from nerve terminals, thus potentiating serotonin neurotransmission.

Co-administration of Li_2CO_3 and milnacipran (3 and 30 mg/kg intraperitoneal) had an additive effect on enhancement of extracellular serotonin levels. Two-way ANOVA with repeated measures indicated a significant interaction between 0.2% Li_2CO_3 and time on extracellular serotonin levels following high-dose (30 mg/kg), but not low-dose (3 mg/kg), milnacipran administration. This interaction suggests the possibility that milnacipran-induced increases in extracellular serotonin concentrations might be more enhanced in lithium-treated rats. However, the overall effects of extracellular serotonin levels were not different between lithium-treated and control rats following high-dose milnacipran administration. Therefore, co-administration of Li_2CO_3 and milnacipran had a similar additive effect on enhancement of serotonin levels, regardless of the dose of milnacipran administered. We previously reported increased extracellular serotonin concentrations following low-and high-dose administration of citalopram (a selective serotonin reuptake inhibitor), which is enhanced by co-administration of Li_2CO_3 in an additive manner (Muraki et al., 2001). Lithium does not inhibit serotonin reuptake (Massot et al., 1999), and chronic Li_2CO_3 treatment increases the number of serotonin transporters in the frontal cortex, as demonstrated by autoradiography (Carli and Reader, 1997). Thus, the additive effect of lithium might be due to increased serotonin neurotransmission, but not the influence on

serotonin transporters. Co-administration of lithium augments the maximum effect of the increases in extracellular serotonin concentrations that are induced by milnacipran. This suggests that the mechanism of lithium augmentation for refractory depression is mediated by increased serotonin neurotransmission beyond that achieved with acute milnacipran alone.

It is possible that lithium-induced increases in extracellular serotonin levels are due to antagonism of serotonin$_{1B}$ receptors. Lithium inhibits the binding of serotonin to serotonin$_{1B}$ receptors in a dose-dependent and non-competitive manner in rats and humans, and the IC_{50} values of lithium for serotonin$_{1B}$ receptors in rats and humans are 0.64 mM and 0.32 mM, respectively (Massot et al., 1999). The serotonin$_{1B}$ receptor is a pre-synaptic autoreceptor. The inhibitory effect of lithium on serotonin$_{1B}$ receptors might intensify serotonin-noradrenaline reuptake inhibitor-induced increases in extracellular serotonin by inhibiting negative feedback. The results of several in vivo microdialysis studies indicate that serotonin$_{1B/1D}$ antagonists increase the effects of selective serotonin reuptake inhibitors on extracellular serotonin concentrations, but that blockade of serotonin$_{1B}$ receptors alone does not increase extracellular serotonin concentrations (Sharp et al., 1991; Gobert et al., 1997). Acute treatment with Li_2CO_3 inhibits activation of serotonin$_{1B}$ receptors (Massot et al., 1999), but does not increase extracellular serotonin levels in the hippocampus (Wegener et al., 2000). Thus, increased basal levels of serotonin cannot be explained by antagonism of serotonin$_{1B}$ receptors by lithium.

In this study, Li_2CO_3 treatment combined with acute low-dose administration of milnacipran significantly elevated extracellular noradrenaline and dopamine levels from baseline values, compared to treatment with low-dose milnacipran alone. These results suggest that treatment with lithium enhances low-dose milnacipran-induced increases in extracellular noradrenaline and dopamine levels. However, treatment with lithium did not enhance the effects of high-dose milnacipran on noradrenaline and dopamine concentrations. We previously reported that the effects of co-administration of Li_2CO_3 and reboxetine, a noradrenaline reuptake inhibitor, on extracellular noradrenaline and dopamine concentrations do not differ from those of reboxetine alone (Kitaichi et al., 2004). Further studies are needed to clarify why lithium enhances the increases in low-dose milnacipran-induced noradrenaline and dopamine concentrations, but not high-dose milnacipran.

The neurochemical effects of Li_2CO_3 and milnacipran in this study are in keeping with some of the behavioural effects of these agents. Alterations in monoamine concentrations after Li_2CO_3 and milnacipran administration may result in a number of functional changes. Li_2CO_3 combined with sub-optimal doses of serotonergic antidepressants reduces immobility time in forced swimming tests in mice (Nixon et al., 1994). The serotonin-noradrenaline reuptake inhibitor venlafaxine is effective in reducing immobility time in forced swimming tests, and co-administration of Li_2CO_3 with sub-optimal doses of venlafaxine reduces immobility time (Redrobe et al., 1998). Increased extracellular concentrations of serotonin might cause the reduction in immobility time when Li_2CO_3 together with sub-optimal doses of serotonin-noradrenaline reuptake inhibitors is administered. Similar to venlafaxine, milnacipran reduces immobility time in a dose-dependent manner in forced swimming tests (Mochizuki et al., 2002), however, the combined effect of Li_2CO_3 and milnacipran on forced swimming tests is not known.

The results of a meta-analysis indicate that augmentation of antidepressant effects by lithium requires at least 7 days of treatment and doses resulting in serum lithium levels of more than 0.5 mEq/l in humans (Bauer and Döpfmer, 1999). In a previous study, we achieved therapeutic plasma lithium levels of 0.71 ± 0.05 mEq/l in rats following the same lithium treatment as that of the present study (0.2% Li_2CO_3 for 7 days) (Muraki et al., 1999). Since co-administration of lithium and serotonin-noradrenaline reuptake inhibitors was observed to increase extracellular serotonin concentrations beyond those observed with serotonin-noradrenaline reuptake inhibitors alone, lithium may increase the antidepressant effects of serotonin-noradrenaline reuptake inhibitors. The combined treatment with Li_2CO_3 and serotonin-noradrenaline reuptake inhibitors might be an effective strategy by which to treat patients with major depressive disorder who are non-responders or partial responders to serotonin-noradrenaline reuptake inhibitors. Recently, in a preliminary study, Kobayashi et al. (2004) suggested that lithium augmentation of milnacipran is effective for the treatment of depression, in which milnacipran is ineffective.

In previous clinical studies of lithium augmentation of antidepressants (Bauer and Döpfmer, 1999), lithium was added to antidepressants that had been ineffective, and lithium addition produced a significant antidepressant effect. However, in clinical practice of the treatment of refractory depression, it is not necessary to follow this procedure that an antidepressant is given for 4–6 weeks, and then lithium is added to the previous antidepressant in nonresponders to this antidepressant. The simultaneous administration of lithium and antidepressants must be effective for refractory depression. Accordingly, in this study, we chose the procedure in that lithium and milnacipran are given to rats simultaneously. For lithium treatment, subchronic lithium treatment for 1 week was chosen because subchronic, but not acute, lithium treatment increases extracellular serotonin concentrations (Wegener et al., 2000). Moreover, there is few study that measured extracellular monoamine concentrations in the rat brain following chronic or subchronic milnacipran administration, and there is no study to investigate the interaction between lithium and serotonin-noradrenaline reuptake inhibitors such as milnacipran on extracellular monoamine concentrations in the rat brain. This is why we chose the treatment procedure of acute

milnacipran following subchronic lithium in this study. Nevertheless, this study has the limitation of the procedure and, in future, it is necessary to examine changes in extracellular monoamine concentrations after chronic milnacipran treatment followed by chronic lithium.

In short, treatment with Li_2CO_3 for 7 days increased basal levels of extracellular serotonin and potentiated serotonin release by milnacipran. These findings suggest that lithium might augment the antidepressant effects of serotonin-noradrenaline reuptake inhibitors.

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