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Subchronic milnacipran treatment increases basal extracellular noradrenaline concentrations in the medial prefrontal cortex of rats

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Abstract

In this study, we investigated the acute effects of milnacipran, a serotonin–noradrenaline reuptake inhibitor, following subchronic treatment with milnacipran (30 mg/kg periorally for 7 days) on extracellular noradrenaline, dopamine and serotonin concentrations in the medial prefrontal cortex. Subchronic administration of milnacipran produced significantly higher basal levels of extracellular noradrenaline. Acute milnacipran administration following subchronic milnacipran treatment for 7 days produced a greater increase in extracellular noradrenaline than a single dose of milnacipran alone. The present results suggest that subchronic milnacipran treatment enhances noradrenergic neural transmission beyond that achieved with acute administration of milnacipran alone, but has no effect on serotonergic or dopaminergic neural transmission.

Keywords: Milnacipran; In vivo microdialysis; Noradrenaline; Dopamine; Serotonin

1. Introduction

Major depressive disorder is a chronic disorder that impairs the social functioning of patients. Antidepressants are clinically effective in the treatment of most patients with major depressive disorder. Recently, serotonin–noradrenaline reuptake inhibitors (SNRIs) were introduced into clinical practice. The antidepressant effect of milnacipran, an SNRI, has been shown to equal that of the tricyclic antidepressants, and to be better tolerated than the tricyclic antidepressants (Kasper et al., 1996; Van Amerongen et al., 2002; Bisserbe, 2002). A number of studies have investigated the mechanism by which SNRIs exert antidepressant effects. Mochizuki et al. (2002) and Koch et al. (2003) have reported that acute SNRI administration increases extracellular serotonin and noradrenaline concentrations in the prefrontal cortex. However, models involving chronic administration of SNRIs might yield more useful data regarding their mechanism of action, since chronic treatment is usually required to achieve remission of depression. Several studies have reported that repeated administration of venlafaxine and duloxetine, other SNRIs, did not alter basal levels of extracellular noradrenaline (Kihara and Ikeda, 1995; Gur et al., 1999; Millan et al., 2001). Chronic administration of duloxetine has been observed to increase extracellular serotonin, but not noradrenaline, over that achieved with single dose duloxetine administration alone (Kihara and Ikeda, 1995). Bel and Artigas (1999) reported that repeated administration of milnacipran did not increase basal concentrations of extracellular serotonin, however, the effect of repeated administration on extracellular noradrenaline and dopamine remains to be determined. Tajima (2002) has reported that milnacipran shows clear signs of efficacy after one week of treatment. In this study, we examined the effect of subchronic milnacipran treatment (30 mg/kg periorally twice daily) on extracellular noradrenaline, dopamine and serotonin concentrations, in the medial prefrontal cortex of rats using an in vivo microdialysis method.

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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 210–300 g, were housed in groups of four and maintained on 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. Experimentation began after 10 days of acclimatization.

2.2. Drugs

Milnacipran (Asahikasei, Japan) was dissolved in distilled water and 1 ml/kg was administered by mouth.

2.3. Microdialysis procedures

Stereotactic implants were placed into the rats under pentobarbital anesthesia (30 mg/kg i.p.) with AG-4 guide cannulae (Eicom, Kyoto, Japan) abutting the surface of the medial prefrontal cortex at the following coordinates relative to the bregma: A+3.2, ML+0.8, and DV+1.0 mm. Dialysis probes with an outer diameter of 0.22 mm (A-I-4-03; Eicom, Kyoto, Japan) were then inserted into the guide cannulae so that 3.0 mm of each probe was exposed to tissue of the medial prefrontal cortex. Rats were then housed in individual cages.

All experiments were performed with freely moving rats. One day following surgery, perfusion with artificial cerebrospinal fluid (145 mM NaCl, 3.0 mM KCl, 1.3 mM $CaCl_2$, and 1.0 mM $MgCl_2$) was started at a flow rate of 1 μl/min. Following an initial perfusion period of 2 h, dialysate samples were collected into sample vials containing 50 μl of 0.05 M acetic acid every 40 min for 480 min. Oral milnacipran was administered 160 min after the first dialysate samples were collected. Extracellular noradrenaline, dopamine and serotonin levels were determined using high-performance liquid chromatography (Eicom, Kyoto, Japan).

Determination of noradrenaline, dopamine and serotonin levels was performed as outlined in a previous paper (Kitaichi et al., 2004).

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.

2.4. Experimental design

The animals were given milnacipran (30 mg/kg) or distilled water by mouth twice daily for 6 days. On day 7, 2 h after the administration of milnacipran (30 mg/kg) or distilled water, stereotactic implants were placed into the rats with guide cannulae abutting the surface of the medial prefrontal cortex, after which dialysis probes were inserted into the guide cannulae. Twenty hours after surgery, perfusion of artificial cerebrospinal fluid was initiated. The rats received a single oral dose of milnacipran (3 or 30 mg/kg) 160 min after the very first dialysate samples were collected.

2.5. Statistical analysis

All data are presented as mean values±S.E.M. of individual rats from each group. Concentrations of noradrenaline, dopamine and serotonin within the dialysate samples are expressed as absolute values (pg/fraction). Repeated measures analysis of variance (ANOVA) for absolute values was used to examine the interaction between 1-week milnacipran treatment and time factors (0–240 min). The average absolute values of five consecutive samples taken prior to milnacipran administration between −160 and 0 min were determined and reported as basal levels. Differences in absolute values measured at each time point of collection between the subchronic milnacipran group and the control group were analyzed using an unpaired *t*-test (two-tailed). Average absolute values were compared with basal levels at each time point of collection using a paired *t*-test. In addition, the area under the curve of acute milnacipran administration was calculated for 0 to 240 min periods in subchronic milnacipran and vehicle treated rats. The area under the curve was analyzed by unpaired *t*-test. Statistical significance was considered $P<0.05$.

3. Results

3.1. Effect of subchronic milnacipran (30 mg/kg for 7 days) treatment on basal levels of extracellular noradrenaline, dopamine and serotonin in the medial prefrontal cortex (Table 1)

Basal extracellular noradrenaline levels measured 24 h after subchronic milnacipran administration for 7 days

Table 1
Effect of subchronic milnacipran treatment (30 mg/kg periorally for 7 days) on basal levels of extracellular noradrenaline, dopamine and serotonin in the medial prefrontal cortex

	Subchronic treatment with distilled water	Subchronic treatment with milnacipran
Noradrenaline	1.132±0.059	2.267±0.207[a]
Dopamine	0.995±0.116	1.084±0.138
Serotonin	3.480±0.477	3.431±0.534

Values represent the mean±S.E.M. (pg/40 min fraction). Data from rats receiving acute challenge doses of 3 and 30 mg/kg milnacipran were pooled. All data were calculated from Fig. 1. Noradrenaline, $N=15$ (control group), $N=16$ (milnacipran group); dopamine, $N=16$; serotonin, $N=16$.

[a] $P<0.001$ vs. controls.

were significantly greater than those observed following subchronic administration of distilled water for 7 days (unpaired *t*-test, $P<0.001$) (Table 1). Basal levels of extracellular dopamine and serotonin were not significantly different between the two groups. Data from rats receiving acute challenge doses of 3 and 30 mg/kg milnacipran were pooled. The data were calculated from Fig. 1.

3.2. Effect of single oral dose administration of milnacipran (3 or 30 mg/kg) on extracellular noradrenaline, dopamine and serotonin concentrations in the medial prefrontal cortex after subchronic milnacipran treatment (Fig. 1)

Acute oral administration of milnacipran (3 and 30 mg/kg) significantly increased extracellular noradrenaline concentrations from baseline levels in control rats and in

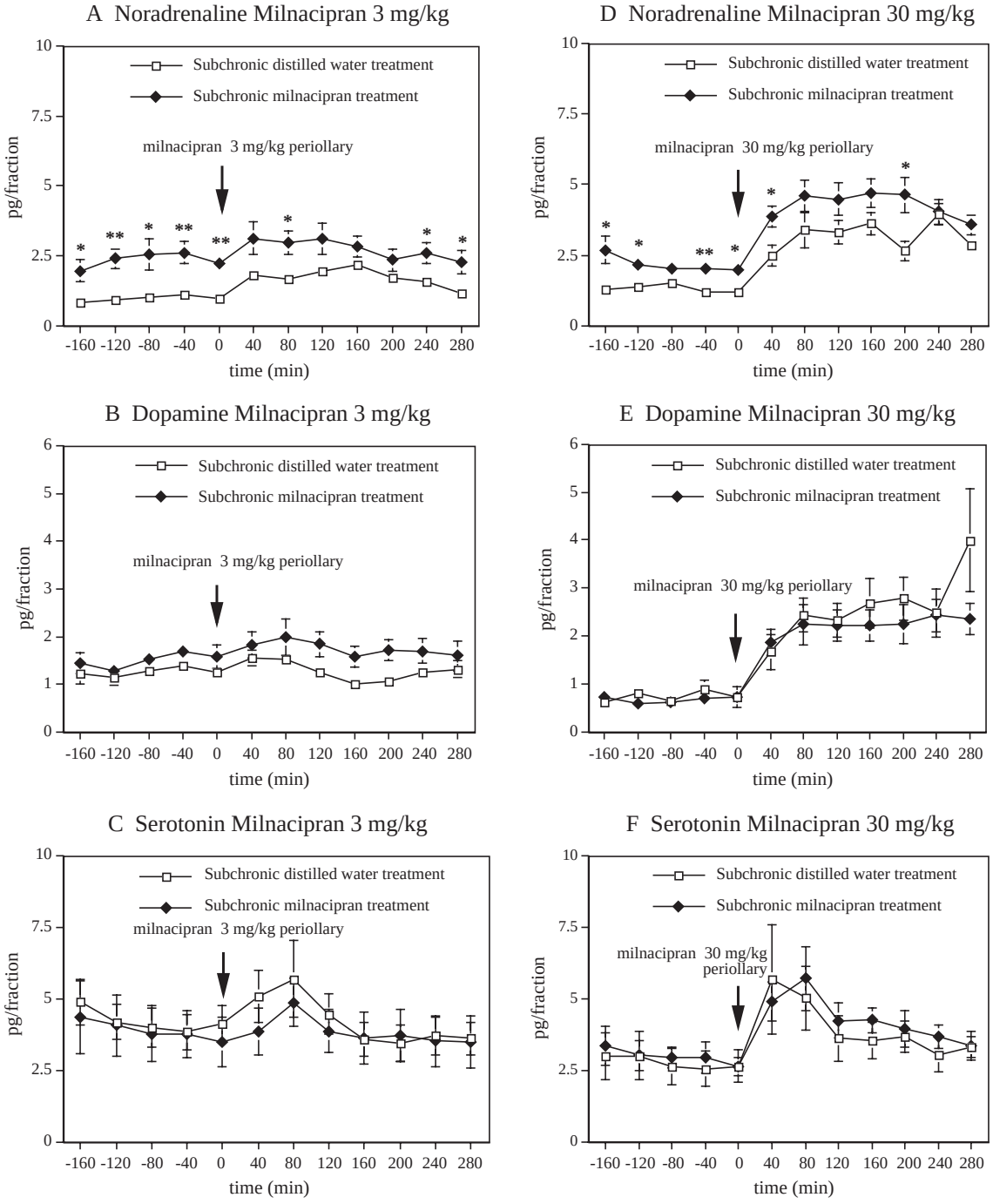

Fig. 1. Effect of single oral dose administration of milnacipran (3 or 30 mg/kg) on extracellular noradrenaline, dopamine and serotonin concentrations in the medial prefrontal cortex of rats after subchronic treatment with oral milnacipran (30 mg/kg for 7 days). Values represent the mean±S.E.M. (pg/40 min fraction). (A) $N=8$; (B) $N=8$; (C) $N=8$; (D) $N=7$ (control group), $N=8$ (milnacipran group); (E) $N=8$; (F) $N=8$. *$P<0.05$, **$P<0.01$, vs. the control group.

rats administered milnacipran for 7 days prior to evaluation [paired *t*-test: control group (administered 3 mg/kg) at 40, 120, 160 and 200 min ($P<0.01$) and at 80 and 240 min ($P<0.05$); control group (administered 30 mg/kg) at all collection points between 120 and 280 min ($P<0.01$), and at 40 and 80 min ($P<0.05$); subchronic milnacipran group (administered 30 mg/kg) at all intervals between 40 and 280 min ($P<0.01$), and at 80 min ($P<0.05$). Two-way ANOVA with repeated measures (0–240 min) indicated significant main effects of subchronic milnacipran treatment [3 mg/kg: $F(1,14)=6.916$ ($P=0.0198$); 30 mg/kg: $F(1,13)=4.906$ ($P=0.0452$)] and time [3 mg/kg: $F(6,84)=5.396$ ($P<0.001$); 30 mg/kg: $F(6,78)=16.144$ ($P<0.001$)] on extracellular noradrenaline concentrations. The interaction between subchronic milnacipran treatment and time was not significant. The subchronic milnacipran group demonstrated significantly greater extracellular noradrenaline concentrations than the control group [unpaired *t*-test: noradrenaline (following administration of 3 mg/kg milnacipran) at −120, −40, and 0 min ($P<0.01$) and at −160, −80, 80, 240, and 280 min ($P<0.05$); noradrenaline (following administration of 30 mg/kg milnacipran) at −40 min ($P<0.01$), and at −160, −120, 0, 40, and 200 min ($P<0.05$)] (Fig. 1A and 1D).

Acute oral administration of milnacipran (3 and 30 mg/kg) in both the control and subchronic milnacipran groups significantly increased extracellular dopamine concentrations from basal levels [paired *t*-test: control group (administered 3 mg/kg) at 40 min ($P<0.01$) and at 80 min ($P<0.05$); control group (administered 30 mg/kg) at all intervals between 80 and 240 min ($P<0.01$) and at 40 and 280 min ($P<0.05$); subchronic milnacipran group (administered 3 mg/kg) at 120 min ($P<0.05$); subchronic milnacipran group (administered 30 mg/kg) at all intervals between 40 and 280 min ($P<0.01$)] (Fig. 1B and 1E). Acute administration of the higher dose of milnacipran (30 mg/kg periollary) also significantly increased extracellular serotonin concentrations from basal levels in both groups [paired *t*-test: control group at 80 min ($P<0.01$) and all intervals between 120 and 200 min ($P<0.05$); subchronic milnacipran group at 40 and 160 min ($P<0.01$) and at 80, 120, and 200 min ($P<0.05$)] (Fig. 1F). Acute administration of the lower dose of milnacipran (3 mg/kg periollary) did not significantly increase extracellular serotonin concentrations from basal levels in either the control or subchronic milnacipran groups (paired *t*-test) (Fig. 1C). Two-way ANOVA with repeated measures (0–240 min) indicated significant effects of time on extracellular dopamine and serotonin concentrations [dopamine (following administration of 3 mg/kg milnacipran): $F(6,84)=4.265$ ($P<0.001$): dopamine (following administration of 30 mg/kg milnacipran): $F(6,84)=13.997$ ($P<0.0001$); serotonin (following administration of 3 mg/kg milnacipran): $F(6,84)=7.335$ ($P<0.0001$) and serotonin (following administration of 30 mg/kg milnacipran): $F(6,84)=7.712$ ($P<0.0001$)]. A significant main effect of subchronic milnacipran treatment on extracellular dopamine and serotonin concentrations was not noted, and the interaction between subchronic milnacipran treatment and time was not significant.

Table 2
Area under the curve of noradrenaline, dopamine and serotonin outputs (during the 0 to 240 min interval) in response to acute milnacipran 3 mg/kg administration

	Subchronic treatment with distilled water	Subchronic treatment with milnacipran
Noradrenaline	425.3±20.8	673.6±97.9 [a]
Dopamine	307.9±21.2	424.4±58.5
Serotonin	1035.0±180.2	934.7±199.1

Values represent the mean±S.E.M. (pg*min). All data were calculated from Fig. 1. Noradrenaline, $N=8$; dopamine, $N=8$; serotonin, $N=8$.
[a] $P<0.05$ vs. subchronic treatment with distilled water.

Table 3
Area under the curve of noradrenaline, dopamine and serotonin outputs (during the 0 to 240 min interval) in response to acute milnacipran 30 mg/kg administration

	Subchronic treatment with distilled water	Subchronic treatment with milnacipran
Noradrenaline	723.9±70.4	1011.8±98.1 [a]
Dopamine	538.9±81.9	494.0±64.2
Serotonin	972.7±213.2	1045.4±141.2

Values represent the mean±S.E.M. (pg*min). All data were calculated from Fig. 1. Noradrenaline, $N=7$ (control group), $N=8$ (milnacipran group); dopamine, $N=8$; serotonin, $N=8$.
[a] $P<0.05$ vs. subchronic treatment with distilled water.

3.3. Effect of single oral dose administration of milnacipran (3 or 30 mg/kg) on the area under the curve of extracellular noradrenaline, dopamine and serotonin concentrations in the medial prefrontal cortex after subchronic milnacipran (Tables 2 and 3)

Compared with the subchronic distilled water group, the area under the curve (0–240 min) of extracellular noradrenaline following acute milnacipran administration (3 and 30 mg/kg) was significantly larger in the subchronic milnacipran group, however, this was not the case for dopamine and serotonin (Tables 2 and 3).

4. Discussion

In this study, subchronic administration of milnacipran (30 mg/kg periollary) for 7 days significantly increased basal levels of extracellular noradrenaline in the medial prefrontal cortex of rats, as measured 24 h after the last dose. Previous studies have indicated that chronic administration of S33005, an SNRI, increases basal levels of extracellular noradrenaline in the frontal cortex of rats (Millan et al., 2001), even though chronic administration of venlafaxine and duloxetine, also SNRIs, has not been found to alter basal levels of extracellular noradrenaline (Kihara and Ikeda, 1995; Millan

et al., 2001). Thus, the effects of chronic administration of SNRIs on basal extracellular noradrenaline levels differ, depending on the SNRI being used. The reasons for this are unclear at the present time.

In the present experiment, single oral dose administration of milnacipran (3 and 30 mg/kg) in control rats following subchronic administration of distilled water significantly increased extracellular noradrenaline concentrations between 40 and 280 min (end point of experimentation) after administration, even though a significant increase in extracellular serotonin was observed between 80 and 200 min following administration of 30 mg/kg. Koch et al. (2003) have reported a more prolonged elevation of extracellular noradrenaline than serotonin following intraperitoneal administration of milnacipran (40 mg/kg). Since they only examined extracellular noradrenaline concentrations over a period of 4 h, it is unclear how long extracellular noradrenaline remains elevated following milnacipran treatment. Thus, acute administration of high-dose milnacipran in vivo causes a more prolonged increase in extracellular noradrenaline than serotonin. On the other hand, high doses of duloxetine and venlafaxine, also SNRIs, have been shown to elevate both extracellular noradrenaline and serotonin 4 h after administration (Koch et al., 2003). These observations might explain why basal extracellular noradrenaline concentrations are increased in the medial prefrontal cortex of rats after subchronic milnacipran treatment.

Daily administration of reboxetine, a selective noradrenaline reuptake inhibitor, over two weeks, has been observed to increase basal extracellular noradrenaline concentrations in the medial prefrontal cortex of rats (Page and Lucki, 2002). Desensitization of presynaptic α_2-adrenoreceptors after chronic treatment with reboxetine (Invernizzi et al., 2001) might be due to increases in basal noradrenaline concentrations. However, no effect of chronic treatment with milnacipran on the function and affinity of α_2 receptors has been observed (Assie et al., 1992; Moret and Briley, 1994). Thus, it is not clear that desensitization of α_2 receptors is related to the increased basal noradrenaline concentrations observed after 7 days of milnacipran treatment in the present experiment.

Previous studies have reported no effect of chronic administration of milnacipran on noradrenaline and serotonin reuptake in rats in vitro (Assie et al., 1992). These findings indicate that it is unlikely that subchronic milnacipran increased basal noradrenaline because of decreased reuptake of noradrenaline following subchronic milnacipran. Moret and Briley (1992) have shown that chronic administration of milnacipran increases the synthesis of both noradrenaline and serotonin. In the present experiment, subchronic milnacipran treatment increased only basal noradrenaline levels and had no effect on basal serotonin concentrations. Thus, it cannot be clearly stated that the observed increase in basal noradrenaline was due to increased synthesis following subchronic milnacipran treatment.

Subchronic milnacipran treatment (30 mg/kg periorally) with acute milnacipran administration (3 and 30 mg/kg) had an additive effect on extracellular noradrenaline concentrations, but not extracellular serotonin or dopamine concentrations. Thus, repeated administration of milnacipran enhances its ability to increase extracellular noradrenaline concentrations in the medial frontal cortex. Similar enhancements of extracellular dopamine and serotonin were not observed, thus, this finding is specific for noradrenaline. On the other hand, chronic administration of duloxetine, an SNRI, has been observed to increase extracellular serotonin, but not noradrenaline, over that achieved with single dose duloxetine administration alone (Kihara and Ikeda, 1995). Therefore, SNRIs (milnacipran and duloxetine) differ with regard to which extracellular monoamine(s) they enhance following subchronic or chronic administration, and this difference may explain differences in the clinical efficacies of various SNRIs.

In conclusion, we investigated the acute effect of milnacipran following subchronic treatment with either milnacipran (30 mg/kg periorally) or distilled water for 7 days on extracellular noradrenaline, dopamine and serotonin concentrations in the medial prefrontal cortex. The subchronic milnacipran group showed significantly greater basal levels of extracellular noradrenaline than the control group. Single dose administration of milnacipran produced a greater increase in extracellular noradrenaline following subchronic milnacipran administration than distilled water. The present study suggests that subchronic milnacipran treatment enhances noradrenergic, but not serotonergic or dopaminergic, neural transmission. This finding suggests that the neurochemical characteristics of milnacipran differ from those of other SNRIs.

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