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Effects of paroxetine or milnacipran on serum brain-derived neurotrophic factor in depressed patients

Reiji Yoshimura *, Masae Mitoma [1], Atsuko Sugita [1], Hikaru Hori [1], Tatsuya Okamoto [1], Wakako Umene [1], Nobuhisa Ueda [1], Jun Nakamura [1]

Department of Psychiatry, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu, Fukuoka 8078555, Japan

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Abstract

Brain-derived neurotrophic factor (BDNF) is an important member of the neurotrophin family of growth factors, abundant in the brain and periphery. Researchers have reported that serum BDNF levels in drug-free depressed patients are lower than those of healthy controls, and have proposed that these low levels might reflect a failure of neuronal plasticity in depression. In the present study, we investigated the effects of paroxetine, an SSRI, and milnacipran, an SNRI, on serum BDNF levels in depressed patients. Serum levels of BDNF were measured by ELISA before, 4 weeks, and 8 weeks after the start of treatment with antidepressants. Forty-two patients were randomly administered paroxetine (21 cases) or milnacipran (21 cases). A negative correlation was found between serum BDNF levels and baseline Ham-D scores. The response and remission rates for each drug were not significantly different. Serum BDNF levels in responders were significantly increased 2.6- and 1.8-fold 8 weeks after treatment with paroxetine or milnacipran, respectively. These results suggest that both drugs improve the depressive state by increasing BDNF levels.

Keywords: Brain-derived neurotrophic factor; Depression; Milnacipram; Paroxetine; Serum

1. Introduction

Depressed patients exhibit pathological changes in particular brain areas, including the limbic (hippocampus and amygdale) and cortical brain regions (Bremner et al., 1995; Mervaala et al., 2000; Manji et al., 2001; Drevetz et al., 1997). Brain-imaging studies of depressed patients indicate impairments in blood flow and decreases in the volume of cortical and limbic structures (Drevetz, 2001; Mervaala et al., 2000). One major neurotrophic factor, brain-derived neurotrophic factor (BDNF), has been found to play a critical role in long-term potentiation, a cellular mechanism of learning and memory, suggesting that this neurotrophic factor can influence neuroplasticity (Figurov et al., 1996; Korte et al., 1995). BDNF is also needed for the survival and guidance of neurons during development and for the survival and function of neurons during adulthood (Duman et al., 2000; McAllister et al., 1999; Radka et al., 1996; Thoenen, 1995). The atrophy and loss of hippocampal or cerebral cortical neurons or glia could result from a stress-induced loss of neurotrophic factors or from other processes that compromise neuronal function and activity or from other insults, as a result of the patient's genetic background (Sapolsky, 2000; Shelton, 2000). There is growing evidence indicating that BDNF may play a crucial role in mental disorders such as depression (Duman et al., 1997, 2000) and schizophrenia (Shoval and Weizman, 2005). Karege et al. (2002) have shown that serum BDNF levels in drug-free depressed patients are lower than those in controls, and Shimizu et al. (2003) found that the serum BDNF levels of treated depressed patients do not differ from control levels. Aydemir et al. (2005) have reported that serum BDNF levels are lower in depressed patients than those in controls, and that treatment with antidepressant drugs for 12 weeks increases serum BDNF levels to control levels. Gonul et al. (2005) have also reported that treatment with several antidepressant drugs for 8 weeks significantly increases serum BDNF levels to the same levels as those of control subjects. These results indicate that antidepressant drugs increase serum BDNF

* Corresponding author. Department of Psychiatry, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu, Fukuoka 8078555, Japan. Tel.: +81 936917253; fax: +81 936924894.
E-mail address: yoshi621@med.uoeh-u.ac.jp (R. Yoshimura).
[1] Tel.: +81 936917253; fax: +81 936924894.

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levels in depressed patients. However, few studies exist comparing selective serotonin reuptake inhibitor (SSRI) with serotonin noradrenaline reuptake inhibitor (SNRI) regarding the influence of serum BDNF levels. We hypothesized that SNRI, which inhibits dual transporters (serotonin and noradrenaline), has greater influence on serum BDNF levels than does SSRI, which inhibits only serotonin transporter. To investigate this hypothesis, we randomly administered paroxetine, an SSRI, or milnacipran, an SNRI, to patients with major depressive disorder, and investigated the effects of paroxetine or milnacipran on serum BDNF levels. We found that both paroxetine and milnacipran equally increase serum BDNF levels, especially in responders to these drugs.

2. Subjects and methods

This study included 42 in- or out-patients in our university hospital who met the DSM-IV-TR criteria for major depressive disorder without psychotic features and who scored at least 16 on the Hamilton Raring Scale for Depression (Ham-D). Fifteen patients were male and 27 were female. The age of the subjects ranged from 28 to 74 years old (mean±SD=47±19). None had received any antidepressant drugs or mood stabilizers at least two weeks prior to the study. All patients were physically healthy and no subjects had a history of alcohol and/or drug abuse. None had co-morbid any anxiety disorders or personality disorders. The patients were randomly divided into either a paroxetine group or a milnacipran group using StatView (Abacus Concepts, Berkeley, CA), a computerized statistical package. There were 21 cases in the paroxetine group, and the remaining 21 subjects were in the milnacipran group. Thirty sex- and age-matched healthy subjects were prepared as a control group (10 male and 20 female, age; mean±SD=45±15 years old). The initial dose of paroxetine or milnacipran was 10 mg/day or 25 mg/day, respectively, and the dose was increased until the patients tolerated adverse effects (the dose was not fixed). The maximum dose of paroxetine or milnacipran was 40 mg/day or 150 mg/day, respectively.

All blood samples were taken at 7:00 am before breakfast (at least 12 h after the last medication) before and 4 and 8 weeks after treatment with paroxetine or milnacipran. Fifteen milliliters of venous blood was drawn with the patient in the supine position, after the patient had been lying at rest overnight. The plasma samples were quickly separated in a centrifuge (2000 *g*, 10 min, 4 °C) and stored at −80 °C until assay. Serum BDNF levels were measured using a BDNF Emax Immunoassay Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. In short, 96-well microplates were coated with anti-BDNF monoclonal antibody and incubated at 4 °C for 18 h. The plates were incubated in a blocking buffer for 1 h at room temperature. The samples diluted with assay buffer 100-times and BDNF standards were kept at room temperature under conditions of horizontal shaking for 2 h, followed by washing with the appropriate washing buffer. The plates were incubated with antihuman BDNF polyclonal antibody at room temperature for 2 h and washed with the washing buffer. The plates were then incubated with anti-IgY antibody conjugated to horseradish peroxidase for 1 h at room temperature, and incubated in peroxidase substrate and tetramethylbenzidine solution to induce a color reaction. The reaction was stopped with 1 mol/L hydrochloric acid. The absorbance at 450 nm was measured with an Emax automated microplate reader. Measurements were performed in duplicate. The standard curve was linear from 5 pg/mL to 5000 pg/mL, and the detection limit was 10 pg/mL. Cross-reactivity to related neurotrophins (NT-3, NT-4, NGF) was less than 3%. Intra- and inter-assay coefficients of variation were 5% and 7%, respectively. The recovery rate of the exogenous added BDNF in the measured plasma samples was more than 95%.

This study was approved by the ethics committee of the University of Occupational and Environmental Health, and written informed consent was obtained from all participants.

Table 1
Demographics for each group

	Paroxetine (21)	Milnacipran (21)	Control (30)
Age	48±25	44±16	45±15
Sex	8/13	8/13	10/20
Number of episodes	2.6±1.4	2.3±1.6	0
Dose	31±13	83±31	0
Baseline Ham-D	24±7	23±6	None

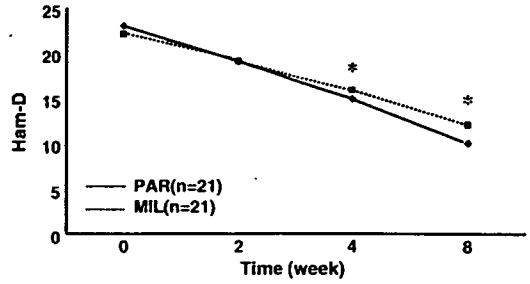

Fig. 1. Changes in Ham-D scores after treatment with paroxetine (—) (F=3.86,*p=0.02: at 4 week) or milnacipran (----) (F=3.37, *p=0.03: at 4 week); compared with time (0 week).

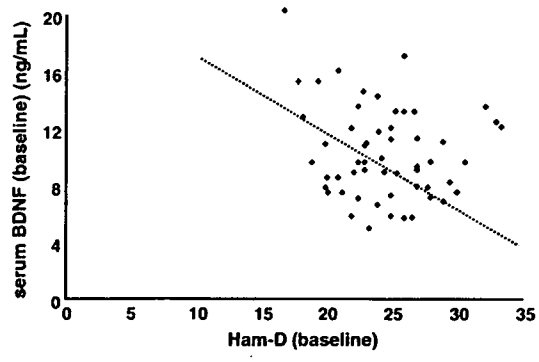

Fig. 2. A negative association between baseline serum BDNF levels and baseline Ham-D scores, r=−0.34, p=0.04

3. Statistical analysis

Statistical analysis was performed by the use of repeated measures of analysis of variance (ANOVA) regarding changes in Ham-D scores and serum BDNF levels in depressed patients. Bonferroni adjustment as a post-hoc comparison was conducted to determine the difference between the groups.

n unpaired *t*-test was performed to compare the whole of depressed patients with the normal controls regarding the serum BDNF levels before antidepressant drug administration. The relationship between two variables was examined using Pearson's correlation coefficients. Significance for the results was set at $p<0.05$.

4. Results

The average daily dosage of paroxetine or milnacipran was 31±13 mg and 83±31 mg, respectively. There were no statistically significant differences between the groups with respect to sex, age, number of depressive episodes, and baseline Ham-D score (Table 1). The Ham-D scores had significantly decreased by 4 weeks after paroxetine or milnacipran treatment (Fig. 1). Fourteen of 21 (67%) in the paroxetine group and 12 of 21 (57%) in the milnacipran group were found to be responders by 8 weeks of treatment with each antidepressant drug. On the other hand, the Ham-D scores of 12 of 21 (57%) in the paroxetine group and 10 of 21 (48%) in the milnacipran group had decreased to less than 7 points (remissional state) by 8 weeks of treatment with each antidepressant drug. No significant difference was found with respect to the response rate or remission rate between groups. There was a negative correlation between serum BDNF levels and baseline Ham-D scores in all 42 depressed patients ($r=-0.34$, $p=0.04$) (Fig. 2). The baseline serum BDNF levels were significantly lower in the whole depressed group than those in the control group ($p=0.02$). However, no correlation was found between age and serum BDNF levels, and serum BDNF levels showed no difference between males and females. In addition, a trend toward negative correlation was found between the suicide scores on the Ham-D and the serum BDNF levels ($r=-0.212$, $p=0.091$). Treatment with paroxetine or milnacipran equally increased serum BDNF levels at 8 weeks, but not 4 weeks. Serum BDNF levels increased 2.6-fold and 1.8-fold in responders to paroxetine and milnacipran, respectively, which was not statistically different in either group. In contrast, serum BDNF levels were not changed in nonresponders to each antidepressant drug. Serum BDNF levels in responders to both drugs at 8 week were higher than those in nonresponders ($p=0.04$) (Table 2).

Table 2
Serum BDNF levels before and 8 weeks after treatment

	0 week (ng/mL)	4 week (ng/mL)	8 week (ng/mL)
Paroxetine (responder)	9.1±7.7	11.6±8.3	22.0±8.5*
Paroxetine (nonresponder)	9.6±8.0	9.9±8.4	13.8±6.7
Milnacipran (responder)	9.9±9.0	10.9±7.9	18.2±9.1*
Milnacipran (nonresponder)	9.6±6.4	9.6±7.2	13.4±7.1
Control	23.4±10.1	None	None

5. Discussion

In the present study, we examined the effects of paroxetine and milnacipran on serum BDNF levels in depressed patients. We found that serum BDNF levels were significantly lower in depressed patients compared to healthy controls; in addition, both paroxetine and milnacipran equally increased serum BDNF levels. Preclinical studies have demonstrated that antidepressant treatments and electroconvulsive seizures increase BDNF expression (Nibuya et al., 1995, 1996; Tapia-Arancibia et al., 2004; Vaidya et al., 1999). Administration of BDNF to stressed animals was reported to produce the antidepressant-like effect of antagonizing learned helplessness (Siuciak et al., 1997). Increased BDNF immunoreactivity in postmortem hippocampal tissue was observed in antidepressant-treated subjects compared with untreated ones (Chen et al., 2001). Dwivedi et al. (2003) reported a decrease in BDNF mRNA and protein levels in the postmortem hippocampus and the frontal cortex of suicide victims, most of whom were diagnosed with major depressive disorder. The findings from these preclinical studies indicate that BDNF plays an important role in the pathogenesis and the treatment of depression. According to clinical studies, Aydemir et al. (2005) reported that treatment with venlafaxine, an SNRI, for 12 weeks significantly increased serum BDNF levels in patients with major depressive disorder and improved to the level of control subjects. Aydemir et al. (2006) also reported that treatment with 10 mg/day of *s*-citalopram, an SSRI, for 6 weeks significantly increases serum BDNF levels in female patients with major depressive disorder. Gervasoni et al. (2005) reported that serum BDNF levels in depressed patients treated with antidepressant drugs (paroxetine, clomipramine, venlafaxine) are significantly increased after remission from their depressive state. Gonul et al. (2005) demonstrated that treatment with SSRIs (fluoxetine, paroxetine, and citalopram) and SNRI (venlafaxine) significantly increased serum BDNF levels to the levels of healthy controls. In addition, Gonul et al. (2005) reported finding no difference between the serum BDNF levels of patients receiving SSRIs or venlafaxine. Basically, our results in the present study, in which paroxetine and milnacipran equally increased serum BDNF levels, were in accordance with the results reported by Aydemir et al. (2006), Gonul et al. (2005) and Gervasoni et al. (2005). Karege et al. (2002) and Gervasoni et al. (2005) reported that serum BDNF levels were significantly decreased in antidepressant-free depressed patients, and that these levels are negatively correlated with the Montgomery–Asberg Depression Rating Scale. Shimizu et al. (2003) have also demonstrated that serum BDNF levels were significantly lower in an antidepressant-naïve group than in either a treated or in a control group, and that there was a significant negative correlation between serum BDNF and Ham-D scores in all patients. Furthermore, they reported preliminary findings that decreased serum BDNF levels in antidepressant-naïve patients recovered to normal levels in association with lower Ham-D scores after treatment with antidepressant medication. In the

present study, a negative correlation was found between serum BDNF levels and baseline Ham-D scores, which was in accordance with the results of studies in Karege et al. (2002) and Shimizu et al. (2003). We also found a trend toward negative correlation between suicide scores on the Ham-D and serum BDNF levels. Recently, Kim et al. (in press) reported that plasma BDNF levels were significantly lower in suicidal depressed patients than in non-suicidal depressed patients, suggesting that reduction of plasma BDNF level is related to suicidal behavior in major depression and that BDNF level may be a biological marker of suicidal depression. Interestingly, although the Ham-D scores decreased significantly by 4 weeks after paroxetine or milnacipran treatment, the serum BDNF levels in responders to the drugs did not change at this point, and the serum BDNF levels significantly increased by 8 weeks after paroxetine or milnacipran treatment. In addition, only responders to paroxetine or milnacipran had increased serum BDNF levels after 8 weeks of treatment with the drugs, while nonresponders to the drugs showed no altered serum BDNF levels from baseline to 8 weeks after the treatment and serum BDNF levels in responders to the drugs were significantly higher than those in nonresponders. These results suggest that serum BDNF levels reflect improvement of the depressive state; in short, that increases of serum BDNF levels might be a biological state marker for recovering from depressive states. Although the precise mechanisms of the increase in serum BDNF levels after 8 weeks of antidepressant treatment remain unknown, enhancement of serotonergic and noradrenergic neurons might be associated with the increase in BDNF levels because of the nonspecificity of the drugs. Although we hypothesized that SNRI has greater influence on serum BDNF levels than does SSRI, this hypothesis was not sustained. Taken together, it is unlikely that the enhancement of noradrenergic neurons in addition to that of serotonergic neurons leads to further increase in the BDNF levels.

In conclusion, baseline serum BDNF levels were significantly lower in the whole depressed group than those in the control group, and treatment with paroxetine and milnacipran for 8 weeks equally brought about a clinical improvement in depressive symptoms, and responders to treatment with these drugs significantly increased the serum BDNF levels to the same levels as the control group, while on the other hand, serum BDNF levels in nonresponders did not. However, our sample in the present study was very small and heterogeneous, and we used a flexible dose regime. Therefore, further studies will be needed to confirm these preliminary findings.

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ORIGINAL ARTICLE

Stimulation of catecholamine synthesis via activation of p44/42 MAPK in cultured bovine adrenal medullary cells by milnacipran

Koji Shinkai · Yumiko Toyohira · Reiji Yoshimura · Masato Tsutsui · Susume Ueno · Jun Nakamura · Nobuyuki Yanagihara

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Abstract Milnacipran is a serotonin noradrenaline reuptake inhibitor (SNRI) and is used clinically as an antidepressant. We report here the effect of milnacipran on catecholamine synthesis in cultured bovine adrenal medullary cells. Incubation of adrenal medullary cells with milnacipran (300 ng/ml, 1,065 nM) for 20 min resulted in a significant increase in ^{14}C-catecholamine synthesis from [^{14}C]tyrosine, but not from [^{14}C]DOPA, whereas the selective serotonin reuptake inhibitors (SSRIs), paroxetine (300 ng/ml, 800 nM) and fluvoxamine (300 ng/ml, 691 nM), had little effect. Milnacipran, but not paroxetine or fluvoxamine, increased the activity of tyrosine hydroxylase, the rate-limiting step of catecholamine biosynthesis, in a concentration-dependent manner (100–300 ng/ml, 355–1,065 nM). U0126 (1 μM), an inhibitor of p44/42 mitogen-activated protein kinase (MAPK) kinase, abolished the stimulatory effects of milnacipran on tyrosine hydroxylase activity. Furthermore, incubation of cells with milnacipran (30–100 ng/ml) for 5 min activated p44/42 MAPK, whereas paroxetine and fluvoxamine did not. The present findings suggest that milnacipran activates tyrosine hydroxylase and then stimulates catecholamine synthesis through a p44/42 MAPK-dependent pathway in cultured bovine adrenal medullary cells.

K. Shinkai · R. Yoshimura · J. Nakamura
Department of Psychiatry, University of Occupational and Environmental Health, School of Medicine, Kitakyushu 807-8555, Japan

Y. Toyohira · M. Tsutsui · S. Ueno · N. Yanagihara (✉)
Department of Pharmacology, University of Occupational and Environmental Health, School of Medicine, Kitakyushu 807-8555, Japan
e-mail: yanagin@med.uoeh-u.ac.jp

Keywords Adrenal medullary cells · Catecholamine synthesis · MAPK · Milnacipran · SNRI · SSRI · Tyrosine hydroxylase

Introduction

Milnacipran, 1-phenyl-1-(diethylaminocarbonyl)-2-(aminomethyl)cyclopropane, is a nontricyclic new antidepressant drug. Milnacipran is a serotonin noradrenaline reuptake inhibitor (SNRI), but it shows preferential inhibition of noradrenaline reuptake when compared to that of serotonin by approximately 2:1 and a lack of effect at any presynaptic receptor (Moret et al. 1985; Stenger et al. 1987). Its antidepressant efficacy has already been established as superior to placebo (Lecrubier et al. 1996) and equivalent to reference tricyclic antidepressants (Kasper et al. 1996). In light of these observations, SNRIs such as milnacipran are regarded as having a mode of action that produces maximal efficacy with an early onset and minimal side effects. Such SNRIs are suggested to be as effective as and better tolerated than tricyclic antidepressants (Montgomery et al. 1996). On the other hand, fluvoxamine and paroxetine are selective serotonin reuptake inhibitors (SSRIs) and have little or no effect on other monoamine reuptake systems. These compounds have little affinity for other neurotransmitter receptors (Tollefson and Rosenbaum 1998), similar to milnacipran.

Adrenal medullary cells derived from embryonic neural crest tissue share many physiological and pharmacological properties with postganglionic sympathetic neurons. Stimulation of the adrenal medulla with acetylcholine, a physiological secretagogue, increases the synthesis of catecholamines, which is closely associated

with the activation of tyrosine hydroxylase, the rate-limiting enzyme of catecholamine biosynthesis (Nagatsu et al. 1964; Weiner 1970). Tyrosine hydroxylase catalyzes the conversion of tyrosine to DOPA, and its activity is acutely regulated by various factors (Zigmond et al. 1989), including enzyme phosphorylation (Cooper et al. 1986). Since the mechanisms of stimulation of catecholamine synthesis and release in adrenal medullary cells are thought to be similar to those of stimulation of noradrenaline synthesis and release in the sympathetic and brain noradrenergic neurons, adrenal medullary cells have provided a good model for the detailed analysis of the action of antipsychotic drugs on catecholamine synthesis, release, and reuptake (Terao et al. 1992; Yoshimura et al. 2001; Shinkai et al. 2005).

A number of studies in the last 30 years have shed light on the effects of classical antidepressants on catecholamine biosynthesis. Previous studies, however, have shown that most of the classical antidepressants do not stimulate catecholamine synthesis. As to the acute effects of tricyclic antidepressants on tyrosine hydroxylase activity, chronic treatment with classical antidepressants has been found to have little effect or an inhibitory effect on the activity or expression of tyrosine hydroxylase in the rat brain in vivo (Leonard 1977; Campbell et al. 1979; Nestler et al. 1990; Moret and Briley 1992).

A previous report (Moret and Briley 1992) showed that acute in vivo administration of milnacipran suppressed the accumulation of DOPA in the rat brain cortex. Recently, we reported that milnacipran and paroxetine but not fluvoxamine competitively inhibit noradrenaline transporter functions in cultured bovine adrenal medullary cells (Shinkai et al. 2005). In the present study, we examined the direct effects of milnacipran on ^{14}C-catecholamine synthesis and tyrosine hydroxylase activity and studied its effect on cellular signaling in cultured bovine adrenal medullary cells, comparing it to that of SSRIs such as fluvoxamine or paroxetine. Unexpectedly, we found that milnacipran, but not fluvoxamine or paroxetine, activates tyrosine hydroxylase and then stimulates catecholamine synthesis, probably through a mitogen-activated protein kinase (MAPK)-dependent pathway in the cells.

Methods

Materials

Oxygenated Krebs-Ringer phosphate (KRP) buffer was used throughout, except when specified. It was composed of the following (in mM): 154 NaCl, 5.6 KCl, 1.1 $MgSO_4$, 2.2 $CaCl_2$, 0.85 NaH_2PO_4, 2.15 Na_2HPO_4 and 10 glucose, adjusted to pH 7.4. Materials were obtained from the following sources: Eagle's minimum essential medium (Eagle's MEM) was from Nissui Pharmaceutical (Tokyo, Japan), collagenase was from Nitta Zerachin (Osaka, Japan), calf serum was from Nacalai Tesque (Kyoto, Japan), Milnacipran hydrochloride was a generous gift from Asahi Kasei (Tokyo, Japan). Fluvoxamine maleate was purchased from Sigma (St Louis, MO, USA). Paroxetine hydrochloride was kindly provided by Smith Kline Beecham (West Sussex, UK). L-[1-^{14}C]tyrosine (54.45 mCi/mmol) was from Perkin Elmer Life Sciences (Boston, MA, USA), L-[U-^{14}C]tyrosine (460 mCi/mmol) and L-[^{14}C]DOPA (6.8 mCi/mmol) were from Amersham Biosciences (Buckinghamshire, UK).

Isolation and culture of bovine adrenal medullary cells

Fresh bovine adrenal glands were used for all experiments. Isolated adrenal medullary cells were obtained by collagenase digestion of slices of adrenal medulla, as reported previously (Yanagihara et al. 1979). The cells were plated at a density of 4×10^6 cells/dish (Falcon 35 mm, Becton Dickinson Labware, Franklin Lakes, NJ, USA) and maintained in monolayer culture in Eagle's MEM containing 10% calf serum, aminobenzylpenicillin (60 μg/ml), streptomycin (100 μg/ml), amphotericin B (0.3 μg/ml) and cytosine arabinoside (3 μM) at 37°C under 5% CO_2/95% air (Yanagihara et al. 1996; Toyohira et al. 2005). The cells were used for experiments after being cultured for between 2 and 7 days.

^{14}C-Catecholamine synthesis from [^{14}C]tyrosine or [^{14}C]DOPA

The cells were incubated with L-[^{14}C]tyrosine (final concentration, 20 μM; 1 μCi) or L-[^{14}C]DOPA (20 μM; 0.25 μCi) in 1.0 ml of KRP buffer in the presence or absence of antidepressants (300 ng/ml) at 37°C for 20 min (for [^{14}C]tyrosine) or 10 min (for [^{14}C]DOPA). The reaction was terminated by aspirating the incubation medium. Then, the cells were harvested in 2 ml of 0.4 M perchloric acid and left standing for more than 30 min in ice to extract the radioactive catecholamines. The precipitated protein was removed by centrifugation at 1,600 g for 10 min, and ^{14}C-labeled catechol compounds in the supernatant were separated further into the fractions containing [^{14}C]dopamine, and [^{14}C]adrenaline plus [^{14}C] noradrenaline by ion-exchange chromatography on Duolite C-25 columns (H^+ type, 0.4×7.0 cm) (Yanagihara et al. 2005). The recovery of adrenaline plus noradrenaline and dopamine by this column was 85.6 and 70.0%, respectively. The ^{14}C-labeled catecholamines were counted in a toluene base scintillator using a liquid scintillation counter (LSC-3500E Aloka, Tokyo, Japan). ^{14}C-Catecholamine synthesis was expressed as the sum of the ^{14}C-catecholamines (adrenaline, nor-

adrenaline and dopamine) because the ratio of [^{14}C] adrenaline plus [^{14}C] noradrenaline/[^{14}C]dopamine was not altered by stimulants.

Tyrosine hydroxylase activity in situ

The cells (10^6 cells/well) (24 well, Falcon) were exposed to 250 µl of the KRP buffer with or without antidepressants, supplemented with 18 µM of L-[1-^{14}C]tyrosine (0.1 µCi) for 10 min at 37°C. Upon addition of L-[1-^{14}C]tyrosine, each well was immediately sealed with an acrylic tube capped with a rubber stopper and fitted with a small plastic cup containing 200 µl of NCS-II tissue solubilizer (Amersham) to absorb the $^{14}CO_2$ released by the cells (Bobrovskaya et al. 1998; Yanagihara et al. 2005). To terminate the reaction, 0.15 ml of 0.8 M perchloric acid was injected into each well through the rubber stopper using a syringe. The plates were left at room temperature for 20–24 h to allow the $^{14}CO_2$ to be absorbed by the NCS tissue solubilizer.

Western blot analysis

The cells were washed three times with 1.0 ml KRP buffer and were pre-incubated in 1.0 ml of KRP buffer at 37°C for 30 min. Then the cells were incubated in 1.0 ml KRP buffer with or without antidepressants (100 ng/ml) for 5 min. They were harvested with 100 µl of a buffer containing 20 mM Tris-HCl (pH 7.5), 5 mM EGTA, 60 mM β-glycerophosphate, 1 mM Na_3VO_4, 0.5% Triton X-100, 6 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, and 20 µg/ml aprotinin. The cell suspension was homogenized and centrifuged at 15,000 g for 30 min. The supernatants were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to an Immobilon-P membrane (Millipore, Bedford, MA, USA), and subjected to immunodetection using anti-phospho-p44/42 MAPK or anti-nonphospho-p44/42 MAPK primary antibodies, horseradish peroxidase-conjugated secondary antibody (horseradish peroxidase-conjugated antibiotin antibody for the protein marker) and Lumi GLO chemiluminescent reagent, as described by PhosphoPlus p44/42 MAPK (Thr202/Tyr204) antibody kit. To measure the proteins, the phospho-p44/42 MAPK and nonphospho-p44/42 MAPK on the membrane were visualized by light-capture and analyzed by CS Analyzer (ver 2.0) (ATTO, Tokyo).

Data analysis

Data are presented as the means ± SEM. The statistical evaluation of the data was performed by ANOVA or Student's *t*-test. When a significant *F*-value was found by ANOVA, Fisher's test for multiple comparisons was used to identify differences among the groups. When $p<0.05$, the differences were considered as statistically significant.

Results

Effect of the antidepressants on ^{14}C-catecholamine synthesis from [^{14}C]tyrosine or [^{14}C]DOPA

The synthesis of ^{14}C-catecholamines from [^{14}C]tyrosine in cells incubated in control medium was found to be approximately linear for up to 30 min (Fig. 1). Milnacipran (300 ng/ml, 1,065 nM) elicited a small but significant ($p<0.05$) increase in ^{14}C-catecholamine synthesis by 22–28% over the control during incubation for 10–30 min. Neither fluvoxamine (300 ng/ml, 691 nM) nor paroxetine (300 ng/ml, 800 nM) increased the synthesis of ^{14}C-catecholamines (Fig. 2a). To ascertain which step in catecholamine synthesis was stimulated by milnacipran, [^{14}C]DOPA was used as a substrate instead of [^{14}C] tyrosine. In this case, however, milnacipran did not alter the synthesis of ^{14}C-catecholamines from [^{14}C]DOPA (Fig. 2b).

Effects of antidepressants on tyrosine hydroxylase activity in the cells

After the cells were treated with the antidepressants (300 ng/ml) or vehicle for 10 min, their tyrosine hydroxylase activity was measured. Milnacipran significantly increased the enzyme activity by 34% over the control

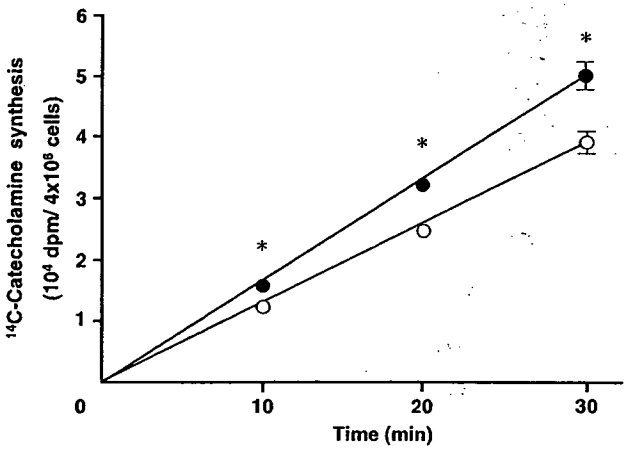

Fig. 1 Time course of ^{14}C-catecholamine synthesis from [^{14}C] tyrosine stimulated by milnacipran in bovine adrenal medullary cells. Cultured cells (4×10^6 cells/dish) were incubated with (*closed circles*) or without (*open circles*) milnacipran (300 ng/ml) at 37°C for indicated times in 1.0 ml KRP buffer containing L-[U-^{14}C]tyrosine (20 µM, 1 µCi). The ^{14}C-labeled catecholamines formed are shown as the total ^{14}C-catecholamines (adrenaline, noradrenaline and dopamine). Data are expressed as the means ± SEM of five experiments carried out in triplicate. *$p<0.05$, compared with control

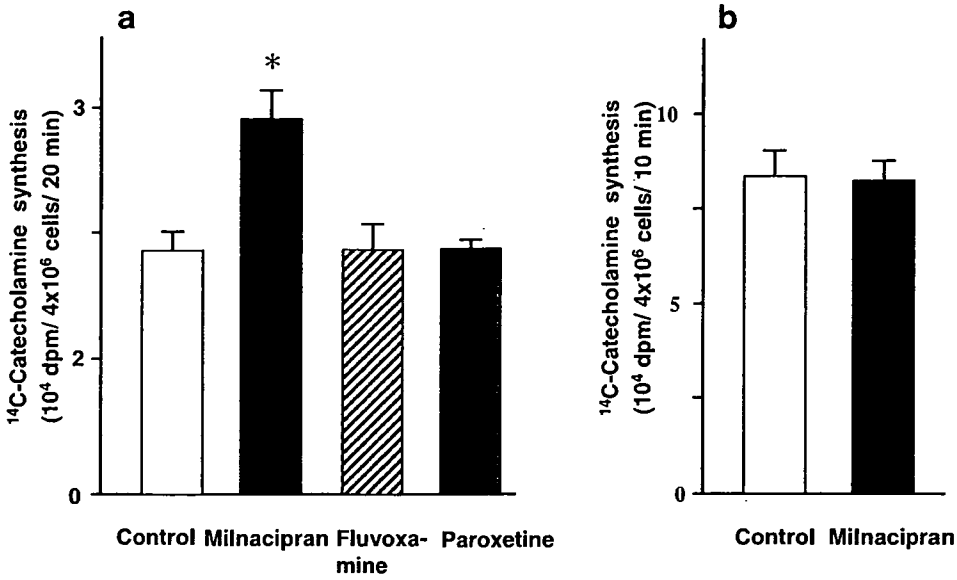

Fig. 2 Effect of antidepressants on ^{14}C-catecholamine synthesis from [^{14}C]tyrosine (**a**) or [^{14}C] DOPA (**b**). **a** The cells were incubated with or without the antidepressant (300 ng/ml) at 37°C for 20 min in the presence of L-[U-^{14}C]tyrosine (20 μM, 1 μCi). **b** The cells were incubated with or without milnacipran (300 mg/ml) at 37°C for 10 min in the presence of L-[^{14}C]DOPA (20 μM, 0.25 μCi). Data are expressed as the means ± SEM of three or four experiments carried out in triplicate. *$p<0.05$, compared with control

(Table 1); neither fluvoxamine nor paroxetine had a significant effect. As shown in Fig. 3, milnacipran significantly increased tyrosine hydroxylase activity in a concentration-dependent manner (100–300 ng/ml, 355–1,065 nM).

Effect of U0126, an inhibitor of MAPK kinase, on tyrosine hydroxylase activity in milnacipran-stimulated cells

Tyrosine hydroxylase is reported to be phosphorylated and activated by p44/42 MAPK (or extracellular signal-regulated protein kinase) (Haycock et al. 1992; Halloran and Vulliet 1994). We examined whether an inhibitor of MAPK kinase alters the stimulatory effects of milnacipran on tyrosine hydroxylase activity in the cells. As shown in Fig. 4, U0126 (1 μM), an inhibitor of p44/42 MAPK kinase, had little effect on the basal activity of tyrosine hydroxylase, but it abolished the stimulatory effects of milnacipran on tyrosine hydroxylase activity.

Activation of p44/42 MAPK in milnacipran-stimulated cells

To further investigate the involvement of MAPK in milnacipran-induced tyrosine hydroxylase activity, we examined the effect of milnacipran on p44/42 MAPK phosphorylation in cultured bovine adrenal medullary cells. Milnacipran (100 ng/ml) caused an increase in phosphorylation of p44/42 MAPK during incubation for 2–10 min (Fig. 5a). A significant increase in p44/42 MAPK phosphorylation

Table 1 Effect of antidepressants on tyrosine hydroxylase activity in the cells

	Tyrosine hydroxylase activity	
	(dpm/10^6 cells/10 min)	(%)
Control	1,750±90	100
Milnacipran	2,330±120*	134
Fluvoxamine	1,780±140	101
Paroxetine	1,680±40	96

The cells were exposed to 250 μl of KRP buffer containing 300 ng/ml antidepressants for 10 min at 37°C in the presence of L-[1-^{14}C] tyrosine (0.2 μCi). Tyrosine hydroxylase activity was measured. Data are expressed as the means ± SEM of four experiments carried out in triplicate. *$p<0.05$ compared with control

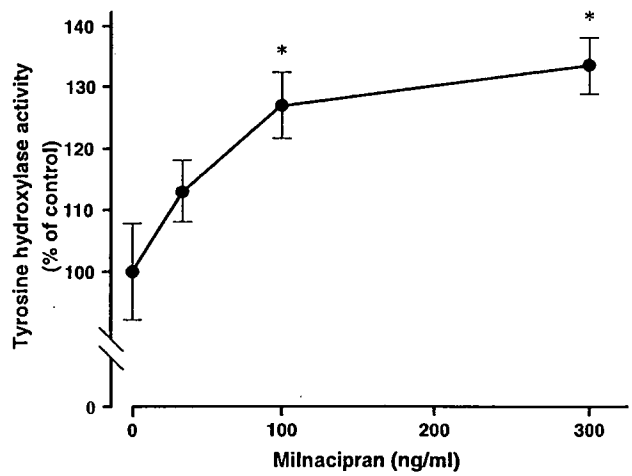

Fig. 3 Concentration-response curve for tyrosine hydroxylase activity induced by milnacipran. The cells were incubated with various concentrations (30–300 ng/ml) of milnacipran at 37°C for 10 min. Data are expressed as the means ± SEM of three experiments carried out in triplicate. *$p<0.05$, compared with milnacipran

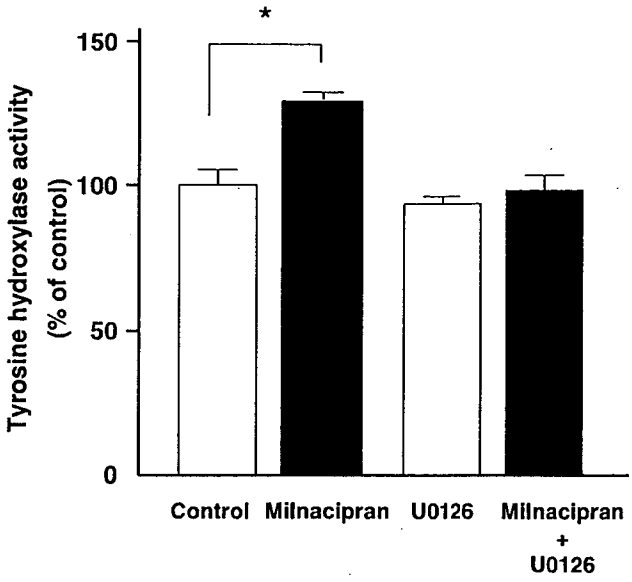

Fig. 4 Effect of U0126, an inhibitor of MAPK kinase, on tyrosine hydroxylase activity in milnacipran-stimulated cells. The cells were incubated with or without milnacipran (300 ng/ml) and/or U0126 (1 μM) at 37°C for 10 min in the presence of L-[1-^{14}C]tyrosine (0.2 μCi). Data are expressed as the means ± SEM of three experiments carried out in triplicate. *$p<0.05$, compared with control

induced by milnacipran was observed at 30–100 ng/ml (Fig. 5b). Furthermore, as shown in Fig. 6, milnacipran significantly increased p44/42 MAPK phosphorylation by 35% and 73% over the control at 5 min, respectively; paroxetine (100 ng/ml, 267 nM) and fluvoxamine (100 ng/ml, 230 nM) had no significant effect.

Discussion

In the present study, we demonstrated that milnacipran, one of the SNRIs, increases ^{14}C-catecholamine synthesis from [^{14}C] tyrosine in cultured bovine adrenal medullary cells. Milnacipran also increased tyrosine hydroxylase activity in the cells. Based on the present findings, it is likely that milnacipran activates tyrosine hydroxylase and subsequently stimulates catecholamine synthesis. To the best of our knowledge, this is the first direct evidence to show the stimulatory effects of milnacipran on catecholamine synthesis.

Several studies have shown that plasma concentrations of milnacipran in human serum reach approximately 135 ng/ml after oral administration of a single dose of 50 mg (Puozzo et al. 1997) and 10–250 ng/ml after oral administration of the

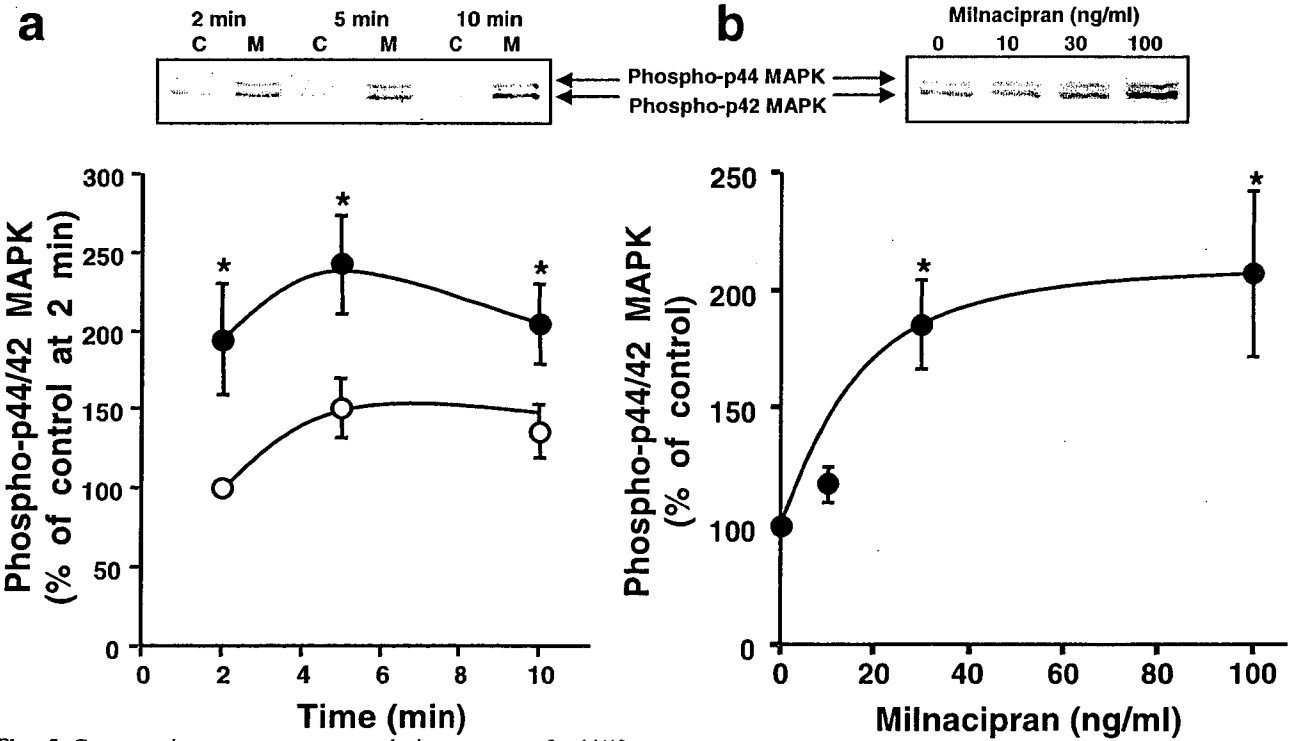

Fig. 5 Concentration-response curve and time course of p44/42 MAPK phosphorylation induced by milnacipran. After preincubation of cells for 30 min at 37°C, the cells were incubated in 1.0 ml KRP buffer with milnacipran (100 ng/ml) (*closed circles*) or vehicle (*open circles*) for indicated times (**a**) and various concentrations of milnacipran (0–100 ng/ml) for 5 min (**b**). *Upper panel*: phospho-p44/42 MAPK. *C* Control, *M* milnacipran. *Lower panel*: data are expressed as the means ± SEM of three experiments and expressed as a percentage of control (the ratio of phospho-p44 plus-p42 MAPK versus non-phospho-p44 plus-42 MAPK). *$p<0.05$, compared to control

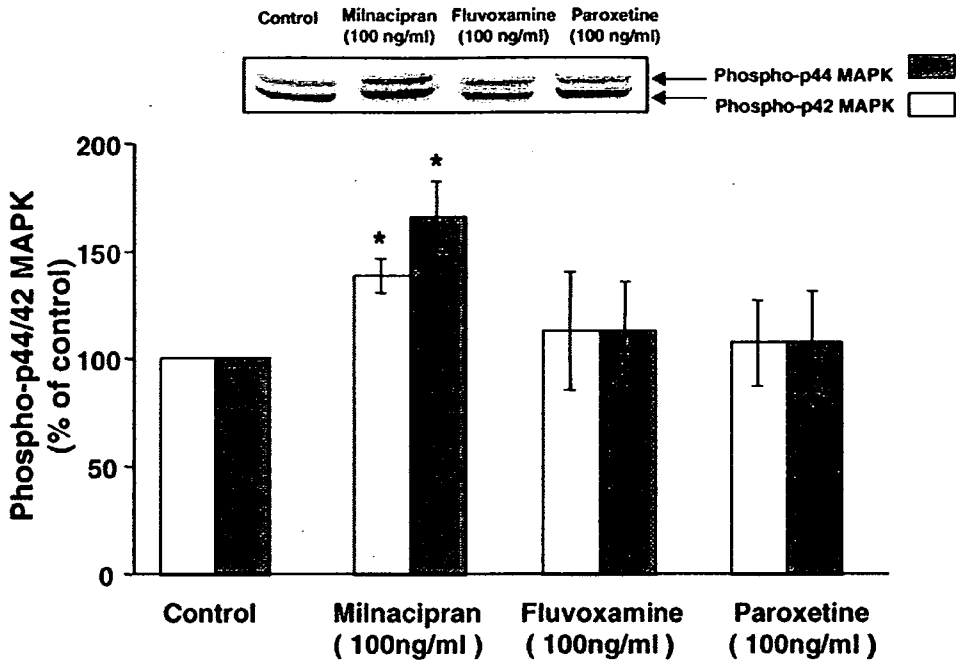

Fig. 6 Effect of antidepressants on phosphorylation of p44/42 MAPK. After preincubation of cells for 30 min at 37°C, the cells were incubated in 1.0 ml KRP buffer with or without milnacipran, paroxetine or fluvoxamine (100 ng/ml) for 5 min. Data are expressed as the means ± SEM of three experiments. *$p<0.05$, compared to control

therapeutic dose in patients with major depressive disorders (unpublished observations). In the present study, milnacipran at 100–300 ng/ml (355–1,065 nM) activated tyrosine hydroxylase in the cells. Therefore, it is likely that the concentrations of milnacipran used in the present study are clinically relevant.

The major question remaining is which receptor(s) and subsequent cellular signal(s) are involved in milnacipran-induced activation of tyrosine hydroxylase. This enzyme is acutely regulated by various factors (for review, see Zigmond et al. 1989), including enzyme phosphorylation (Cooper et al. 1986). Several experiments have shown that tyrosine hydroxylase can be phosphorylated and activated by multiple protein kinases (Zigmond et al. 1989), including p44/42 MAPK (Haycock et al. 1992; Halloran and Vulliet 1994).

On the other hand, there is growing evidence that the MAPK pathway represents a novel target for the development of improved therapeutics for depression. Mercier et al. (2004) reported that fluoxetine rapidly activates MAPK cascades in rat astrocytes and that genes involved in neuroprotection are induced in an MAPK-dependent or independent manner. Duman et al. (2006) also showed a role for MAPK signaling in behavioral models of depression and antidepressant treatment.

In the present study, the stimulatory effect of milnacipran on tyrosine hydroxylase activity was nullified by U0126, an inhibitor of MAPK kinase, suggesting that milnacipran increases the activity of tyrosine hydroxylase through an MAPK-dependent pathway. To confirm this possibility, we further examined whether treatment of the cells with antidepressants enhanced the phosphorylation of p44/42 MAPK. Milnacipran significantly increased p44/42 MAPK phosphorylation at 30–100 ng/ml, which seems to be slightly more sensitive than that for activation of tyrosine hydroxylase, whereas paroxetine and fluvoxamine had little effect on both responses. These findings suggest that stimulation of tyrosine hydroxylase activity induced by milnacipran is mediated at least in part via the activation of p44/42 MAPK.

The second question is which receptor(s) participates in the activation of MAPK in the cells. Milnacipran has been reported to be devoid of interactions at any known neurotransmitter receptor or ion channel (Briley et al. 1996), although a recent study showed that milnacipran at high concentrations inhibited ligand-gated ion channels, such as nicotinic acetylcholine receptors (Ueta et al. 2004). This is in agreement with a previous report (Hennings et al. 1999), suggesting that monoamine uptake inhibitors share the same action mechanism with nicotinic acetylcholine receptors, that is, they bind into the ion channel of the receptors. Furthermore, milnacipran does not act at adrenergic, muscarinic cholinergic, or histamine receptors (Briley et al. 1996).

A previous report has shown that noradrenaline activates extracellular-regulated kinase (p44/42 MAPK) in primary cerebral cortical cultures of rat brains (Tolbert et al. 2003). This might give rise to the possibility that milnacipran inhibits noradrenaline transporters, which, in turn, causes an accumulation of noradrenaline in the medium and

activates MAPK in the cells. This possibility, however, may not be viable for the following reasons. Paroxetine is an SSRI, but therapeutic concentrations of paroxetine inhibit noradrenaline transporters in the rat brain (Owens et al. 2000). Indeed, in our recent study (Shinkai et al. 2005), the concentration of paroxetine used in the present study (100 ng/ml) also inhibited [^{3}H]noradrenaline uptake by 45% of control in bovine adrenal medullary cells, but did not increase catecholamine synthesis and MAPK phosphorylation. Therefore, the stimulatory mechanism of milnacipran remains to be determined, and further investigation will be required to clarify this mechanism.

Several lines of evidence have shown that in vivo administration of classical tricyclic antidepressants, such as imipramine and desipramine, and of the new antidepressant milnacipran inhibits the synthesis of noradrenaline in rat brains, and that the inhibition of noradrenaline synthesis closely parallels the inhibition of noradrenaline reuptake (Carlsson and Lindqvist 1978; Moret and Briley 1992). Moret and Briley (1992) have suggested that the firing of noradrenergic neurons by milnacipran-induced noradrenaline reuptake inhibition is decreased through a feedback system, such as the actions of noradrenaline on somatodendritic α_2-adrenoceptors. This is well supported by several other papers (Eisenhofer et al. 1991; Huangfu et al. 1995) in which desipramine-induced decrease in noradrenaline release was partially mediated by an action of raised intrasynaptic noradrenaline concentrations on inhibitory α_2-adrenoceptors.

In the present study, milnacipran stimulated catecholamine synthesis and tyrosine hydroxylase activity in cultured cells. Thus, there seems to be a discrepancy in the effects of milnacipran on catecholamine synthesis between the in vivo results in rat brains and our present findings. This discrepancy may be related to the fact that: (1) our present results should be reflected by the direct effect of milnacipran on catecholamine synthesis in the cells; (2) the amount of milnacipran (30–300 mg/kg) used in the study of Moret and Briley (1992) was quite high compared to the amounts used clinically and (3) divergent effects of noradrenaline transporter inhibitors such as desipramine are reported to be shown by differential sympathoadrenal actions, characterized by inhibition of noradrenaline release from sympathetic nerves and stimulation of adrenaline secretion from the adrenal glands (Eisenhofer et al. 1995). Therefore, further in vivo experiments are required to examine the effect of clinically relevant doses of milnacipran on catecholamine synthesis in rat brains. In the present study, milnacipran stimulated MAPK and catecholamine synthesis. In light of the treatment of depressed patients, it is noteworthy that milnacipran has unique effects related to the stimulation of MAPK and catecholamine synthesis in addition to the inhibition of noradrenaline reuptake.

Recent studies reported that polymorphism of the noradrenaline transporter, but not the serotonin transporter, in part determines the antidepressant response to milnacipran (Yoshida et al. 2004) and that noradrenaline-deficient mice lack responses to antidepressants (Cryan et al. 2004), supporting that the brain noradrenergic neurons play an important role in the clinical effects of antidepressants (Delgado et al. 1993). Indeed, the present findings that milnacipran stimulates catecholamine synthesis would be consistent with our recent clinical observations that depressed patients with low levels of plasma 3-methoxy-4-hydroxy-phenylglycol (MHPG), a major metabolite of brain noradrenaline, tend to respond to treatment with milnacipran and that there is a positive correlation between increases in plasma MHPG levels and clinical improvements after administration of milnacipran (Shinkai et al. 2004). Although the stimulatory effects of milnacipran on catecholamine synthesis might be related in part to the clinical observations, further in vivo study will be required to examine this possibility.

In conclusion, we have demonstrated that milnacipran activates tyrosine hydroxylase activity and subsequently stimulates catecholamine synthesis via activation of MAPK in vitro. The present findings add a new pharmacological action to our understanding of the treatment of depressed patients with milnacipran.

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Case report

Serotonin syndrome in a case of depression with various somatic symptoms: The difficulty in differential diagnosis

Takeshi Terao *, Takatoshi Hikichi

Department of Neuropsychiatry, Oita University Faculty of Medicine, Hasama-machi, Oita 879-5593, Japan

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Abstract

A 65-year-old female patient with major depressive disorder suffered from clonus, shivering and impaired visual acuity after 20 mg/day of paroxetine administration. The symptoms were initially regarded as further manifestations of her somatic symptoms of depression, and paroxetine was increased to 30 mg/day resulting in frequent clonus, increased shivering, serious dysarthria, ongoing impairment in visual acuity and agitation. These symptoms subsided upon paroxetine discontinuation. Ten mg/day of paroxetine rechallenge provoked dysarthria, tremor and headache, but these symptoms improved again upon paroxetine discontinuation. These findings indicate that the patient's symptoms were not somatic in origin but were in fact the symptoms of serotonin syndrome. In conclusion, the present case suggests the difficulty in diagnosing serotonin syndrome in a patient with somatic symptoms.

Keywords: Depression; Paroxetine; Serotonin syndrome; Somatic systoms

1. Introduction

Serotonin syndrome is a potentially life-threatening adverse drug reaction that results from therapeutic use, intentional self-poisoning, or inadvertent interactions between drugs (Kojima et al., 1993). Although serotonin syndrome is often described as a clinical triad of mental-status changes, autonomic hyperactivity and neuromuscular abnormalities, not all of these signs consistently present in all patients with this syndrome (Boyer and Shannon, 2005). Moreover, it is sometimes difficult to determine if a patient with somatic symptoms is exhibiting new somatic symptoms or has in fact developed serotonin syndrome whilst taking therapeutic doses of selective serotonin reuptake inhibitors (SSRIs).

In this report, we describe a patient of depression with various somatic symptoms who showed symptoms of serotonin syndrome which had been regarded as somatic.

2. Case report

A 65-year-old unmarried woman, who was very much involved in her chosen hobby as a wood engraver, was working towards a pressing deadline when her brother was admitted to a hospital. These stressors lead to palpitation and a stiff shoulder for which she initially sought the help of a chiropractor and whom she subsequently visited three times. She began to develop muscle weakness in the right lower limb and called the chiropractor for advice but was disappointed by his poor response. Her symptoms were worsening gradually over the ensuing three months and by the time she saw me she was experiencing a depressive mood, loss of interests, hypochondriacal tendency, tension, anxiety, sleep disturbance (difficulty in falling asleep, shallow sleep, and early awakenings), weight loss and particularly aches in her neck and shoulders. She also complained of muscle weakness in both lower and upper limbs, leading to gait disturbance. The patient had already seen a neurosurgeon who indicated to her that there were no abnormalities of her brain or neck on MRI. She had never been in a depressive state. Thus, according to DSM-IV-TR, she was diagnosed as suffering from major depressive disorder, single episode. Particularly, her somatic symptoms were prominent among her complaints.

* Corresponding author. Tel.: +81 97 586 5823; fax: +81 97 549 3583.
E-mail address: terao@med.oita-u.ac.jp (T. Terao).

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The patient was receiving 0.5 mg/day of etizolam and 10 mg/day of zolpidem from the previous doctor. In combination with these drugs, she was also taking 80 mg/day of valsartan, 120 mg/day of verapamil, 100 mg/day of aspirin plus 2 mg/day of warfarin, 180 mg/day of loxoprofen, and 150 mg/day of teprenone in the treatment of hypertension, arrhythmia, angina pectoris, lumbago, and chronic gastritis, respectively. On neurological examination, a slight hyperreflexia at the patellar tendon was noted but there were no other abnormalities.

Ten mg/day of paroxetine was started in combination with the aforementioned drugs. A week after starting paroxetine she found that she could walk more easily than before. Paroxetine was increased to 20 mg/day, but one week later she complained of reduced visual acuity and particularly of spontaneous clonus in her lower limbs at night and shivering over her body when she was tired. She reported that the clonus and shivering symptoms subsided if she was lying down on her bed. Despite these complaints, she was nevertheless happy as her gait had become much improved. Because her complaints were regarded as further manifestations of her somatic symptoms probably due to depression at that time, paroxetine was further increased to 30 mg/day. Three days later (i.e., 17 d after starting 20 mg/day of paroxetine), she called me in an agitated state and complained of frequent clonus. Her speech was impaired by a marked dysarthria, so much so that it was very difficult to understand what she was saying. I instructed her to discontinue her paroxetine immediately and four days later she came to see me. By then, there was no evidence of agitation, and her clonus and dysarthria had much improved. Also, her visual acuity had increased. With her paroxetine stopped, she continued with the other medications.

In the 2 weeks following paroxetine discontinuation, the patient did not experience clonus or dysarthria but her anxiety increased. Whilst 20–30 mg/day of paroxetine had brought abovementioned symptoms, there had been no adverse side effects while taking 10 mg/day of paroxetine. As such, we decided to restart paroxetine at 10 mg/day with the provision that if she suffered from similar symptoms on this dosage, she should discontinue the paroxetine. Three days later, she was forced to discontinue paroxetine again because the dysarthria recurred. She also noticed tremor and headache. Four days after paroxetine discontinuation, her tremor and headache improved.

3. Discussion

The present case suffered from clonus, shivering, dysarthria, impaired visual acuity and agitation after 20–30 mg/day of paroxetine administration. Although these symptoms were initially regarded as further manifestations of her somatic symptoms, they subsided upon paroxetine discontinuation. Ten mg/day of paroxetine rechallenge provoked dysarthria, tremor and headache and these also improved upon paroxetine discontinuation. These findings suggest that these symptoms were not somatic symptoms of depression but were actually the symptoms of serotonin syndrome. The first episode fully met both the diagnostic criteria by Sternbach (1991) and also that of Boyer and Shannon (2005) whereas the second episode met criteria partially. Boyer and Shannon (2005) suggest that clonus (inducible, spontaneous, and ocular) are the most important features in establishing the diagnosis of serotonin syndrome. In the current case, clonus was also prominent but for a while both clonus and impaired visual acuity were regarded as somatic symptoms with depression. Actually, impaired visual acuity might have been due to mydriasis, which is one of serotonin syndrome symptoms.

Although approximately 15% of overdoses of SSRIs exhibit serotonin toxicity (Gillman, 2006), there are a few reports of serotonin syndrome induced by therapeutic doses of SSRI's (Mills, 1995; Kaneda et al., 2002; Ochiai et al., 2003). Mills (1995) reported an 18-year-old man developed serotonin syndrome 2 h after receiving his second dose of paroxetine (20 mg/day). Kaneda et al. (2002) reported a 23-year-old woman presenting serotonin syndrome after a single dose of paroxetine 20 mg. Ochiai et al. (2003) showed a 48-year-old man with serotonin syndrome after 6 d of 20 mg/day of paroxetine. In contrast to these cases which shows a rapid development of serotonin syndrome in patients with paroxetine treatment, our patient took paroxetine for 24 d and then developed serotonin syndrome. This delay may be another reason why her symptoms were not regarded as ones of serotonin syndrome. On the other hand, there is a possibility that many paroxetine-induced serotonin syndromes (particularly mild ones) have been overlooked because it is difficult to differentially diagnose somatic symptoms which are due to her/his mental disease or serotonin syndrome, such as in the present patient.

Although our patient took other drugs in the course of her paroxetine treatment, these were unchanged throughout the two episodes of serotonin syndrome. Naturally, it is possible that some of these drugs interacted with paroxetine and precipitated the development of serotonin syndrome in this patient. Regrettably, although it is uncertain whether such interaction brought about serotonin syndrome or which concomitant drug actually interacted with paroxetine in this case, it seems important to emphasize that serotonin syndrome is often precipitated by unrecognized drug interactions. Additionally, it should be noted that this case had slight hyperreflexia before paroxetine administration. As hyperreflexia is generally accepted as one of the symptoms of serotonin syndrome, slight hyperreflexia before SSRI administration could be a predictor of vulnerability to serotonin syndrome.

In conclusion, the present case suggests the difficulty in diagnosing serotonin syndrome in a patient with various somatic symptoms of depression.

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I am pleased to inform you that your manuscript has been accepted for publication in The Primary Care Companion. I am forwarding it to the publisher for final editing, and you will hear from them in a few months regarding a publication date. Congratulations to you and your co-authors!

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A Letter to the Editor

Small Doses of Aripiprazole Augmentation of Antidepressants: Three Case Reports

Takeshi Terao, MD, PhD
Department of Neuropsychiatry
Oita University Faculty of Medicine

Correspondence: Takeshi Terao, MD, PhD
Professor of Neuropsychiatry
Oita University Faculty of Medicine
Oita 879-5593, Japan

e-mail: terao@med.oita-u.ac.jp

Several studies showed the usefulness of aripiprazole augmentation of antidepressants in refractory depression[1-4]. Berman et al[4] suggest that the effective dose for many (depressive) patients is lower than those recommended for schizophrenia and bipolar disorder and that the true efficacious dose for some patients may have been lower. In most cases, however, aripiprazole was started at 5-10 mg/day and increased to 10-30 mg/day[1-3]. Probably, smaller dose may be useful to prevent side effects and thereby reduce drop out. There is, to my knowledge, no report describing small doses of aripiprazole augmentation and maintenance.

In the present study, I report three cases of refractory depression responding to 3 mg/day of aripiprazole and maintain remission with the same dose. This study was approved by the university ethics committee and written informed consent was obtained from the three patients.

Case 1

Ms A was a 61-year-old female with major depressive disorder prolonged for 2 years. Just before aripiprazole augmentation, she was receiving 150 mg/day of fluvoxamine for 2 months and her Hamilton Rating Scale for Depression (HAM-D) score was 19. Twelve days after 3 mg/day of aripiprazole addition, her HAM-D score improved to 6. After 5 months of remission, she complained of insomnia and aripiprazole was discontinued whereas fluvoxamine was continued. Within 2 weeks, depression relapsed and aripiprazole was resumed. Two weeks later, her condition improved and subsequently she was in remission for 5 months.

Case 2

Mr B was a 46-year-old male with major depressive disorder prolonged for 7 years. Just before aripiprazole augmentation, he was receiving the combination of 150 mg/day of amoxapine, 100 mg/day of sertraline, 50 mg/day of trazodone and 30 mg/day of mianserin for 7 weeks and his HAM-D score was 14. Two weeks after adding 3 mg/day of aripiprazole, HAM-D score improved to 5. After 6 months of remission, he was restored to junior high school teacher. During another 6 months, he could gradually adjust himself to his job with the same drugs.

Case 3

Ms C was a 27-year-old female with major depressive disorder prolonged for 3 years. Just before aripiprazole augmentation, she was receiving the combination of 40 mg/day of

paroxetine, 100 mg/day of maprotiline, 800 mg/day of lithium for 4 weeks and her HAM-D score was 21. One week after adding 3 mg/day of aripiprazole, HAM-D score dramatically improved to 0. Thereafter, lithium and paroxetine were discontinued without relapse. After 5 months of remission, she began to work as a clerk. During another 5 months, she could gradually adjust herself to her job with 100 mg/day of maprotiline and 3 mg/day of aripiprazole.

These cases responded to 3 mg/day of aripiprazole augmentation very well and the effects were maintained for several months without increasing aripiprazole dosage. Particularly in case 1, aripiprazole withdrawal induced relapse and resumption did remission. Although placebo effects cannot be ruled out completely, these findings suggest that small doses of aripiprazole addition may be useful for some refractory patients. Further controlled trials are required to draw a definite conclusion.

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Changes in regional cerebral blood flow following antidepressant treatment in late-life depression

Junko Ishizaki[1], Hideki Yamamoto[1], Taro Takahashi[1], Maki Takeda[1], Madoka Yano[1,2] and Masaru Mimura[1]*

[1]*Department of Neuropsychiatry, Showa University School of Medicine, Tokyo, Japan*
[2]*Department of Psychology, Keio University, Tokyo, Japan*

SUMMARY

Objective Reversible/irreversible abnormalities of regional cerebral blood flow (rCBF) are seen in patients with depression. However, in late-life depression there is little evidence of a longitudinal change in rCBF through remission. We examined whether the decreased rCBF in individuals with late-life depression resolves following treatment.

Methods Twenty-five depressed patients older than 55 years completed the Hamilton Rating Scale for Depression and single photon emission computed tomography before and after a mean of 13.7 weeks of pharmacotherapy. Quantitative analyses were performed using the Statistical Parametric Mapping procedure.

Results Patients with depression demonstrated decreased rCBF in the anterior ventral and dorsal medial prefrontal cortex (PFC), including anterior cingulate cortices, bilateral ventrolateral PFC to temporal cortices, and bilateral medial to lateral parieto-occipital lobes relative to healthy controls. No particular areas showed increased rCBF. Following pharmacotherapy, rCBF significantly increased in the left dorsolateral PFC to precentral areas and the right parieto-occipital regions. However, decreased rCBF at baseline in the anterior ventral/dorsal medial PFC, bilateral ventrolateral PFC, bilateral temporal lobes, and bilateral parietal lobes did not show significant improvement after treatment.

Conclusions Remarkable improvements in rCBF in the left dorsolateral PFC to precentral regions are consistent with the hypothesis that neuronetworks including the left frontal cortex may be functionally and reversibly involved in late-life unipolar major depression (state-dependent). In contrast, neural circuits including bilateral medial, dorsolateral, and parietal areas may reflect underlying and continuous pathognomonic brain dysfunction of depression (trait-dependent).

KEY WORDS—vascular depression; late-life; SPECT; frontal lobe

INTRODUCTION

A number of functional neuroimaging studies, including positron emission tomography (PET) and single photon emission computed tomography (SPECT), demonstrate focal or diffuse cerebral dysfunction in individuals with depression. On one hand, studies have documented an overall decrease in hemispheric mean cerebral blood flow (CBF) and/or rate of glucose metabolism in depression (Lesser *et al.*, 1994; Kocmur *et al.*, 1998). On the other hand, studies report a decrease/increase in regional CBF (rCBF) and/or glucose metabolism in particular regions of the brain. Such regions include the prefrontal cortex (PFC), cingulate, amygdala/hippocampus, basal ganglia, or thalamus, suggesting involvement of the networks that regulate mood and emotion (Buchsbaum *et al.*, 1986; Curran *et al.*, 1993; Dolan *et al.*, 1993; Bench *et al.*, 1995; Lesser *et al.*, 1994; Goodwin, 1997; Mayberg *et al.*, 1999; Nobler *et al.*, 2000; Anand and Shekhar, 2003; Navarro *et al.*, 2004). Although results of studies are inconsistent, most research focuses on dysfunction of mood-related circuits involving the multiple prefrontal cortices and limbic structures. For example, Baxster *et al.* (1989) used PET and reported

*Correspondence to: Dr M. Mimura, Department of Neuropsychiatry, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8666, Japan.
E-mail: mimura@med.showa-u.ac.jp

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that glucose metabolic rates of patients with depression were significantly lower than those in healthy controls in the left anterior dorsolateral PFC. Mayberg *et al.* (1994) reported that patients with severe unipolar depression demonstrated significant CBF decreases in the bilateral frontal cortices, anterior temporal cortices, anterior cingulate cortices (ACC), and caudate compared with healthy controls. The greatest decrease was seen in the paralimbic regions, specifically in the inferior frontal lobe and ACC.

Much less is known concerning the longitudinal changes in CBF/glucose metabolism in depression. Some studies demonstrate that the decrease in rCBF observed during a depressive episode is reversible and recovers to a level comparable with healthy subjects, suggesting that changes in rCBF are disease-state dependent (Baxter *et al.*, 1989; Goodwin *et al.*, 1993; Mayberg *et al.*, 1994; Buchsbaum *et al.*, 1997; Brody *et al.*, 1999; Mayberg *et al.*, 1999, 2000, 2002). Bench *et al.* (1995) reported patterns of change in rCBF following clinical remission of depression. Remission was associated with a significant increase in rCBF in the left dorsolateral and medial PFC, including the ACC. Researchers have also suggested that unmedicated subjects with depression showed increased rCBF/metabolism, which decreased with effective antidepressant treatments (Buchsbaum *et al.*, 1997; Drevets, 1999; Mayberg *et al.*, 1999).

On the other hand, decreased rCBF observed in the anterior PFC and left amygdala areas has been shown to remain at a lower level compared with healthy subjects, suggesting trait-dependent abnormalities (Drevets *et al.*, 1992). Recent functional activation studies using a transient mood challenge revealed that patients in remission from depression still show rCBF decreases in the pregenual ACC (Liotti *et al.*, 2002). These persistent decreases may suggest a potential depression trait marker independent of clinical illness status.

Additionally, elderly patients with depression may frequently show multiple small lacunae/cerebrovascular lesions. In 1997, Krishnan *et al.* and Alexopoulos *et al.* proposed the term 'vascular depression' to categorize this subtype of depression occurring in the context of cerebrovascular disease. Although most CBF studies of depression have been confined to depression without cerebrovascular changes (Goodwin *et al.*, 1993; Bench *et al.*, 1995), the above-mentioned network activities would likely be enhanced in vascular depression secondary to frontal-subcortical disconnections. Individuals with late-life and young depression have been reported to show similar longitudinal time-course changes of rCBF in general. However, areas showing rCBF improvement are inconsistent. Navarro *et al.* (2002) found that the left frontal hypoperfusion in elderly depressed patients disappeared during remission; supporting the hypothesis that neuronetworks involving the left frontal cortex may be functionally and reversibly involved in late-life unipolar major depression. Kimura *et al.* (2003) reported the result that in vascular depression left anterior frontal rCBF was lower in both depressed and remitted states compared to non-vascular depression. This finding might not only represent a trait marker, but also correlate with the duration of disease and likelihood of recurrence and relapse.

The main aim of our study is to make sure where in brain CBF abnormality would change and assess the relation between essential brain abnormalities and depressive symptoms with late-life depression.

METHODS

Participants

Twenty-five elderly inpatients or outpatients (3 men and 22 women) aged 55 years or older (age = 70.4 ± 7.5 years) were included in this study. All the patients were right-handed. Inclusion criteria were Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria for major depressive disorder. No one had a history of any other major psychiatric disorders. In addition, patients with neurological diseases including apparent cerebrovascular events and degenerative disorders were excluded.

Table 1. Demographic variables and characteristics of the participants

	$N = 25$
Sex, female %	88.0
Education, years	11.4 (3.5)
Age, years	
Onset of the first depressive episode	62.0 (12.0)
Time at examination	70.4 (7.5)
Number of depressive episodes	2.4 (2.0)
Single episode %	44.0
Pharmacotherapy %, Equivalent imipramine mg/day	
Paroxetine	32, 104.8 (39.5)
Milnacipran	40, 94.9 (54.3)
Tricylic antidepressants	28, 112.2 (56.4)
HRSD score	22.6 (6.0)
MMSE score	27.0 (2.8)
Hachinski score	3.7 (1.6)

Data are expressed as mean (SD) unless otherwise indicated.
HRSD = Hamilton Rating Scale for Depression; MMSE = Mini-Mental State Examination.

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