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*SHORT COMMUNICATION*

# Differential effects of diazepam, tandospirone, and paroxetine on plasma brain-derived neurotrophic factor level under mental stress

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**Objectives** Serum brain-derived neurotrophic factor (BDNF) levels are reduced in depressed patients, and successful antidepressant treatment leads to increases in BDNF levels. However, little is known about how psychotropic drugs affect the mechanism of the human response to mental stress. We investigated the influence of psychotropic drugs on plasma BDNF levels under mental stress using a driving simulator (DS) task.
**Methods** Fourteen healthy male volunteers received one of four drugs, diazepam (5 mg), tandospirone (20 mg), paroxetine (10 mg), and matched placebo, in a double-blind, crossover manner. Subjects were asked to perform the DS task 4 h post-dosing. Plasma BDNF levels were measured before and after the DS task.
**Results** Plasma BDNF levels under the placebo, diazepam, and tandospirone conditions significantly decreased after the DS task compared with before the task. Conversely, no significant differences in plasma BDNF levels were detected under the paroxetine condition.
**Conclusion** As these three psychotropic drugs have differential effects on plasma BDNF levels under mental stress after 4 h post-dosing, antidepressants, unlike anxiolytics, might have a prompt positive effect on the mental stress response. 

KEY WORDS—antidepressant; anxiolytic drug; brain-derived neurotrophic factor; mental stress

## INTRODUCTION

Stress is common in everyday life and is believed to affect happiness, health, and cognition (Caspi *et al*., 2003). A role for brain-derived neurotrophic factor (BDNF) in the effects of stress and the response to antidepressant treatment is supported by studies demonstrating opposing regulation of this neurotrophic factor (Charmey, 2004). BDNF, the most abundant neurotrophin in the brain, enhances the growth and maintenance of several neuronal systems and serves as a neurotransmitter modulator (Shimizu *et al*., 2003). BDNF is present in blood and can pass through the blood–brain barrier carried by a high-capacity, saturable transport system (Pan *et al*., 1998). Although the source and function of blood BDNF remains unknown, recent reports have shown that more than 99% of blood BDNF proteins are stored in platelets and can be released in serum (Radka *et al*., 1996) and that blood levels of BDNF might in part reflect BDNF levels in the brain (Karege *et al*., 2002, Mitoma *et al*., 2008).

The "neurotrophin hypothesis of depression" is based largely on two observations: a decrease in hippocampal BDNF levels is correlated with stress-induced depressive behavior, and antidepressant treatment enhances the expression of BDNF (Martinowich *et al*., 2007). Recent studies suggested that serum BDNF levels are reduced in depression (Sen *et al*., 2008, aan het Rot *et al*., 2009). Antidepressants are thought to upregulate the expression of BDNF and its receptor and to promote adult neurogenesis, which might be the core pharmacological effect of antidepressants (Martinowich and Lu, 2008); successful antidepressant treatment leads to an increase in plasma BDNF levels (Lee and Kim, 2008).

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Stress can decrease the expression of BDNF in the hippocampus (Duman and Monteggia, 2006). However, little is known about how psychotropic drugs affect the human response to mental stress. In our previous study, we examined the effects of antidepressants and anxiolytic drugs using driving simulator (DS) tasks (Iwamoto *et al*., 2008, Takahashi *et al*., 2010). Here, we adapted the DS task as the psychological stressor in order to examine how mental stress influences plasma BDNF levels and to investigate the effect of psychotropic drugs on plasma BDNF levels under mental stress conditions.

## MATERIAL AND METHODS

Fourteen healthy male volunteers (32–44 years old, mean ± SD, 37.2 ± 3.6 years) were included. All subjects had had a driving license for at least 10 years and regularly drove a car for a minimum of 5000 km per year. Health interviews and the Structured Clinical Interview for DSM-IV conducted at the time of the study indicated that none of the participants had any physical or psychiatric disorders. The study was approved by the Nagoya University Graduate School of Medicine and Nagoya University Hospital ethics review committee, and written informed consent was obtained from each subject prior to participation.

The schedule of this study is shown in Figure 1. The study was a double-blind, placebo-controlled, crossover study with four periods of treatment, each separated by a washout period of at least 7 days. Each subject was assigned to receive four treatments in a randomized, counterbalanced order set by laboratory personnel, who did not test subjects and analyze results. The random allocation sequence of each subject was concealed until the study termination. During each treatment period, the subjects received a single dose of each of the study drugs: diazepam (5 mg), tandospirone (20 mg), paroxetine (10 mg), and matched placebo. The doses selected were based on general clinical recommendation for starting dose. All treatments were supplied in identical capsules for the double-blind design.

Each subject took one of the four drugs at 11:00 AM. The DS task was conducted 4 h after drug administration when the plasma concentration of paroxetine reaches its maximum (Doyle *et al*., 1989, Ghose, 1989). Blood samples (10 mL) were collected in anticoagulant tubes before and after the DS task. Blood sample was immediately centrifuged at 1700 g for 10 min, and plasma sample was stored at −30 °C until used. Plasma BDNF levels were determined by enzyme-linked immunosorbent assay (Promega Co., Madison, WI, USA).

The car-following task in the DS task was used as the mental stressor. The details about this simulator (Toyota Central R&D Labs, Inc., Japan) are available elsewhere (Uchiyama *et al*., 2003, Iwamoto *et al*., 2008). The weighted average scores [adaptive weighted workload (AWWL)] (Miyake and Kumashiro, 1993) in the abridged Japanese version of the National Aeronautics and Space Administration Task Load Index (NASA-TLX) (Haga and Mizukami, 1996) was used to evaluate the mental stress of the car-following task. Seventeen healthy male volunteers completed the following two mental stress tasks using the DS in random order. One was a standard driving task, which required the subjects to drive the car freely on the road, and another was the car-following task that required the subjects to maintain a constant distance between the cars without the discretion of subjects. The time needed for the completion of both tasks is 5 min. The subjects were asked to rate the NASA-TLX after finishing each task, and the AWWL scores for each condition were calculated for subsequent analysis.

Statistical differences were determined with the paired *t*-test. Significance levels were set to 5% for all tests.

## RESULTS

The AWWL scores for the car-following task condition were significantly higher than the normal driving task condition (mean ± SD: 55.2 ± 16.9 vs. 38.2 ± 20.8; $p < 0.01$).

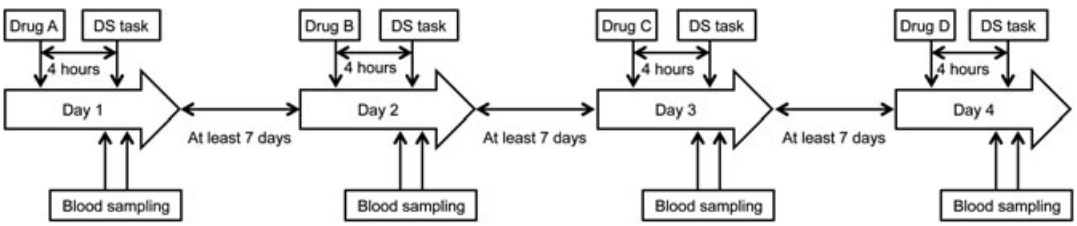


Figure 1. The figure shows the schedule of the study. Days 1, 2, 3, and 4 are treatment periods; each is separated by a washout period of at least 7 days. During each treatment period, the subjects received a single dose of one of the study drugs (drugs A, B, C, and D ): diazepam (5 mg), tandospirone (20 mg), paroxetine (10 mg), and matched placebo. Each subject took one of the four drugs at 11:00. The DS task was conducted 4 h after drug administration. Blood samples were collected before and after the DS task



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The effects of psychotropic drugs on plasma BDNF levels before and after the car-following task are shown in Figure 2. Under the placebo condition, plasma BDNF levels after the car-following task were significantly decreased compared with the plasma BDNF levels before the task (mean ± SD: $0.64 \pm 0.31$ vs. $0.34 \pm 0.21$, $p < 0.01$). We also found that under the diazepam and tandospirone conditions, plasma BDNF levels after the car-following task were significantly decreased compared with plasma BDNF levels observed before the task (mean ± SD: $0.49 \pm 0.23$ vs. $0.34 \pm 0.21$, $p < 0.05$ and mean ± SD: $0.59 \pm 0.36$ vs. $0.31 \pm 0.14$, $p < 0.01$, respectively). Conversely, these changes were not observed under the paroxetine condition (mean ± SD: $0.57 \pm 0.27$ vs. $0.79 \pm 0.63$, $p = 0.19$).

## DISCUSSION

From the AWWL scores, we considered the car-following task as a mental stress condition. In the present study, we investigated the effect of psychotropic drugs on plasma BDNF levels under mental stress using a DS task as the stressor. Although the task associated with increased mental stress significantly decreased plasma BDNF levels under the diazepam, tandospirone, and placebo condition, the same effect was not observed under the paroxetine condition.

Regarding psychological stress, a previous study of healthy subjects demonstrated that levels of perceived mental stress in the workplace were inversely correlated with serum BDNF levels (Mitoma *et al*., 2008). Both acute and chronic mental stress may reduce serum BDNF levels. According to these findings, mental stress might negatively affect stress-vulnerable depressed patients in whom serum BDNF levels are already decreased.

A previous report indicated that antidepressants could enhance BDNF gene expression by activating cyclic adenosine monophosphate response element binding protein (Martinowich and Lu, 2008). Furthermore, a recent study showed that antidepressants directly promote BDNF release from platelets in rats (Watanabe *et al*., 2010). Considering that plasma BDNF levels did not significantly decrease under mental stress following acute administration of paroxetine in our result, paroxetine might promote short-term (several minutes) BDNF release from platelets in human models, although further examination would be needed.

Although anxiolytic drugs such as diazepam and tandospirone can relieve stress-related symptoms, there are no reports indicating that anxiolytic drugs influence plasma BDNF levels. One study showed that stimulation of the gamma-aminobutyric acid system (i.e., diazepam) in adult Wistar rats results in an immediate decrease in hippocampal BDNF mRNA levels (Zafra *et al*., 1991). To our knowledge, the effects of diazepam on plasma BDNF levels have not been examined in humans. The present results suggest that benzodiazepine had no influence on plasma BDNF

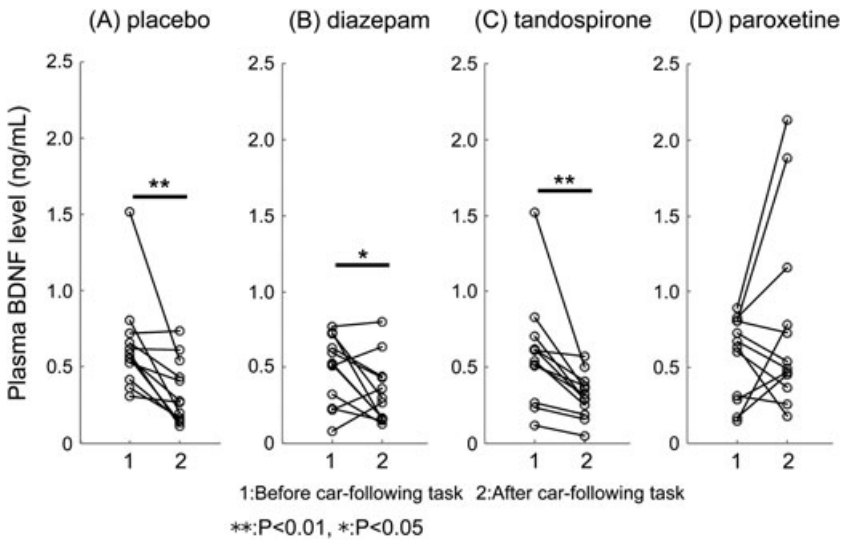


Figure 2. The effects of psychotropic drugs on plasma BDNF levels before and after the car-following task. Panel (A) shows the change in plasma BDNF levels during the placebo condition. Plasma BDNF levels after the car-following task are significantly decreased compared with levels before the task ($p$ 0.01). Panels (B) and (C) show plasma BDNF levels following diazepam and tandospirone conditions. Plasma BDNF levels are significantly decreased after the car-following task compared with levels before ($p < 0.05$ and $p < 0.01$, respectively). Panel (D) shows plasma BDNF levels under the paroxetine condition. There is no significant difference in plasma BDNF levels before and after the car-following task ($p = 0.19$)



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levels immediately following stress. In terms of the "neurotrophin hypothesis of depression," antidepressants, but not anxiolytic drugs, can ameliorate the symptoms of depression and prevent stress-related recurrence of depression.

The present study has several limitations. First, the sample was restricted to a small number of healthy adult male volunteers. It is possible that the responses to mental stress in female, depressed, or elderly patients could differ widely from those of healthy, younger men. Second, the present study evaluated only the immediate effects of low-dose administration of the drugs on plasma BDNF levels. Third, a 5-min simulator task may be inadequate for assessing mental stress. Although AWWL scores showed that this task induced mental stress, there is a possibility of a type 1 error because of the small sample size. Therefore, future studies using a larger number of subjects with repeated drug administration over a range of doses need to be conducted for conclusions to be drawn regarding the effects on plasma BDNF level. From the AWWL score, we regarded the car-following task as a mental stress condition, although there is no significant difference in plasma cortisol levels before and after the car-following task (data not shown). Then it is necessary to examine how the duration of the DS task influences plasma BDNF levels in more detail. Fourth, the degree of stress associated with the DS task needs to be examined by measuring changes in other stress-related variables (e.g., heartbeat and skin electrical resistance). Fifth, we did not examine plasma BDNF level change at 4 h post-dosing without DS task to elucidate whether drug treatments without DS task could affect plasma BDNF levels. Finally, we evaluated only 4-h time point for DS task when plasma concentration of paroxetine reaches its maximum. Because three drugs have different pharmacokinetic and pharmacodynamic profiles, we need to examine plasma BDNF level change at a time when plasma concentrations of diazepam and tandospirone reach their maximum in future study.

Our findings should be interpreted with following caveat. The treatments for depression, such as antidepressants (Shimizu *et al*., 2003), electroconvulsive therapy (Okamoto *et al*., 2008), and sleep deprivation (Gorgulu and Caliyurt, 2009) increase expression of BDNF. Although this is suggesting that there is an etiological link between the development of depression and BDNF, scientific studies have found that numerous brain areas show altered activity in depressed patients (Krishnan and Nestler, 2008), and it has not been possible to determine a single cause of depression.

In conclusion, diazepam, tandospirone, and paroxetine could have different effects on plasma BDNF levels under mental stress after 4 h post-dosing. Furthermore, antidepressants, unlike anxiolytics, might have immediate positive effects on the mental stress response.

## CONFLICT OF INTEREST

None declared.

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