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Fluvoxamine, a selective serotonin reuptake inhibitor, suppresses tetrahydrobiopterin levels and dopamine as well as serotonin turnover in the mesoprefrontal system of mice

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Abstract *Rationale:* Tetrahydrobiopterin (BH₄) is a coenzyme of tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH), rate-limiting enzymes of monoamine biosynthesis. According to the monoamine hypothesis of depression, antidepressants will restore the function of the brain monoaminergic system, and BH₄ concentration. *Objective:* To investigate the effects of fluvoxamine on BH₄ levels and dopamine (DA) and serotonin (5-HT) turnover in the mesoprefrontal system, incorporating two risk factors of depression, social isolation and acute environmental change. *Methods:* Male ddY mice (6W) were divided into two housing groups, i.e. group-housing (eight animals per cage; 35 days), and isolation-housing (one per cage; 35 days), SC injected with fluvoxamine (20 or 40 mg/kg; days 29–35), and exposed to 20-min novelty stress (day 35). The levels of BH₄, DA, homovanilic acid (HVA), 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) were measured in the prefrontal cortex and midbrain. *Results:* Under the group-housing condition, novelty stress significantly increased BH₄ levels in both regions, and the HVA/DA ratio in the midbrain, whereas it did not change any parameters in either region under the isolation-housing condition. In the prefrontal cortex, fluvoxamine significantly decreased the 5-HIAA/5-HT ratio under the group-housing condition, and BH₄ levels and the HVA/DA ratio under the isolation-housing condition. In the midbrain, fluvoxamine significantly decreased all parameters, except for an increasing in the 5-HIAA/5-HT ratio under the isolation-housing condition. *Conclusion:* Isolation-housing suppressed the increase of BH₄ levels and DA turnover elicited by novelty stress. Fluvoxamine

suppressed BH₄ levels, and DA and 5-HT turnover. Fluvoxamine may have altered DA turnover by suppressing BH₄ levels.

Keywords Social isolation · Novelty stress · Animal model · Tetrahydrobiopterin · Dopamine turnover · Serotonin turnover

Introduction

(6R)-5,6,7,8-tetrahydrobiopterin (BH₄) is a coenzyme of tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH), which are the rate-limiting enzymes of monoamine biosynthesis. BH₄ is also a coenzyme of NO synthase (NOS). NO is known to act as a signaling molecule in the central nervous system (CNS) (Barañano et al. 2001; Kiss and Vizi 2001; Ohkuma and Katsura 2001; Prast and Philippu 2001; Esplugues 2002). Thus, BH₄ plays an important role in CNS activity.

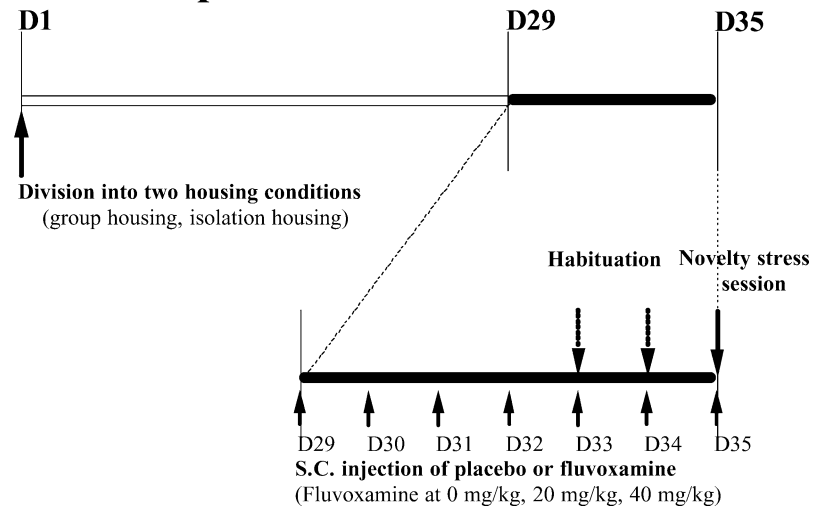
With regard to the cause of human depression, the monoamine hypothesis focuses on impaired function of the monoaminergic system of the brain (Smith et al. 1997; Delgado 2000; Hirschfeld 2000; Leonard 2000). Pharmacological studies of almost all clinically effective antidepressants have supported this hypothesis. Antidepressant-induced suppression in the activity of TH (Nestler et al. 1990) and TPH (Lapierre et al. 1983) may be related to BH₄ levels. Furthermore, recent studies suggest the possibility that alteration of NOS activity may be related to the antidepressant-like effects of NOS inhibitors in animal models (Harkin et al. 1999; Karolewicz et al. 1999; Da Silva et al. 2000). Investigation into the relationship between changes in the activities of these enzymes (TH, TPH, and NOS) and BH₄ levels induced by antidepressants should therefore help to clarify the pathophysiology of human depression.

Concerning environmental risk factors of depression, most patients become ill after adverse life events, such as interpersonal loss (separation, etc.) (Paykel 1994). Further, absence of social support appears to be associated with an

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Fig. 1 Experimental time schedule. Mice were divided into 12 groups as described in Materials and methods

Experimental time schedule



onset and relapse of depression (Paykel 1994). Kendler showed that genetic factors cooperate with environmental factors to induce the onset of depression in humans (Kendler et al. 1993). Thus, we proposed an animal model that simulated two of the major environmental risk factors of human depression (Miura et al. 2002a,b; Miura et al. 2004), i.e. social isolation and acute environmental change (Kendler et al. 1993; Paykel 1994).

We propose that changes in brain BH₄ levels play an important role in the pathophysiology of depression, and that antidepressants modulate these changes. Our recent study suggested that fluvoxamine, an SSRI, suppressed BH₄ levels as well as 5-HT turnover in the hippocampus of mice (Miura et al. 2004). In the present study, we further investigated other regions that we suspect are involved in the pathophysiology of human depression, and we measured BH₄, dopamine (DA), and serotonin (5-HT) levels simultaneously. Mesocorticolimbic DA projections (A8, A10) originating from the ventral tegmental area (VTA) of the midbrain (Cooper et al. 2003) have been shown to play an important role in a reward system, i.e. in motivating behavior (Kupferman and Schwartz 1995). We therefore selected two regions of focus, the prefrontal cortex and the midbrain. The aim of the present study was to investigate the effects of fluvoxamine on BH₄ levels and DA and 5-HT turnover in the mesoprefrontal regions, and to clarify the role of BH₄ in our novel animal model simulating two of the major environmental risk factors of human depression (Miura et al. 2002a,b, 2004).

Materials and methods

Animals

A total of 96 male ddY mice were used in the present experiments. The mice were transported from a breeding company at 5 weeks of age to our experimental animal center. After a 1-week habituation period, the mice, all of which had previously been housed in groups (eight per

cage), were divided into two different groups according to housing conditions, i.e. a group-housing group (eight per cage; $n=48$) and an isolation-housing (one per cage; $n=48$; Fig. 1) group. The cages used for group-housing were 21×31×13 cm, and the cages used for isolation-housing were 17×29×13 cm. After being assigned to one of the two housing conditions, the mice were reared for 35 days (Fig. 1). Cage exchange was performed twice a week. Food and water were provided ad libitum. A 12-h light/dark cycle was maintained and room temperature was maintained at 21–23°C. All efforts were made to minimize both the number of animals used and the degree of their suffering. All of the experiments were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). The experiments also comply with the current laws of Japan.

Fluvoxamine injection

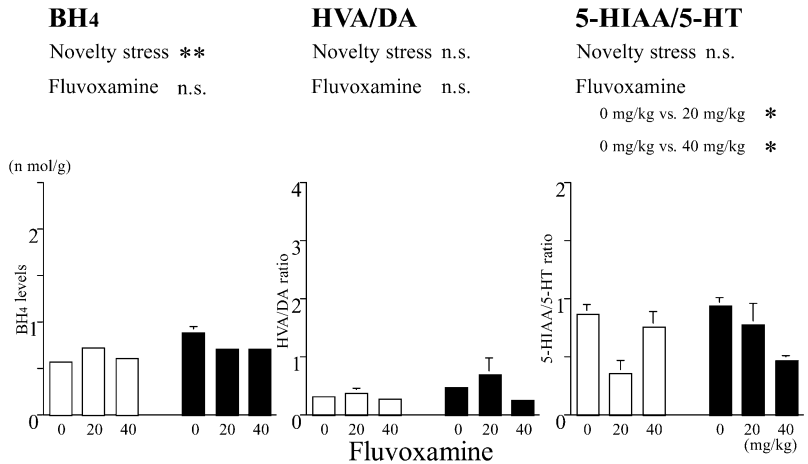
In week 5 (days 29–35) after being assigned to one of the two housing conditions, the mice were SC injected with placebo (distilled water) or low-dose (20 mg/kg) or high-dose (40 mg/kg) fluvoxamine once per day (Fig. 1). The fluvoxamine was kindly donated by Solvay Pharmaceuticals (Brussels, Belgium). The mice were then further divided into three groups: a control (0 mg/kg, $n=32$), a low-dose (20 mg/kg, $n=32$), and a high-dose (40 mg/kg, $n=32$; Fig. 1) group.

Novelty stress test

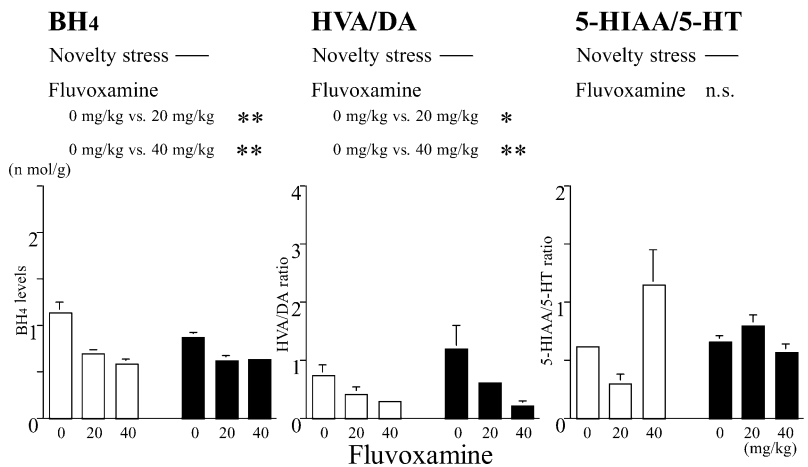
After being assigned to one of the two housing conditions, the animals were further separated into two groups: a stress group ($n=48$) in which the animals were exposed to a 20-min novelty stress on day 35 [i.e. the animals were placed into a transparent plastic box (28×35×30 cm) that they had not yet experienced]; and a non-stress group ($n=48$) in which the animals experienced a habituation

Fig. 2 Changes in BH₄ levels, and HVA/DA and 5-HIAA/5-HT ratios in the prefrontal cortex elicited by novelty stress and by fluvoxamine. **A** group-housing condition ($n=48$); **B** isolation-housing condition ($n=47$). *White bars*, non-stress ($n=48$); *black bars*, novelty stress ($n=47$, $n=95$ total). Fluvoxamine: 0.0 mg/kg ($n=31$); 20.20 mg/kg ($n=32$); 40.40 mg/kg ($n=32$, $n=95$ total). Each *bar* indicates the final group division. The number of animals used for each group was eight, except in the case of the isolation-housed, stress, 0 mg/kg group ($n=7$). Values are shown as the mean \pm SEM. *Asterisks* indicate the results of the Tukey-Kramer test for novelty stress and fluvoxamine in each housing and stress condition: * $P<0.05$, ** $P<0.01$, *n.s.* not significant. In the isolation-housing condition, the post hoc test for novelty stress was not performed because the MANOVA result was not significant

A Group housing



B Isolation housing



session (i.e. the animals were placed into the transparent plastic box for 10 min on days 33 and 34 before the 20-min session on day 35; Fig. 1). The habituation session was performed in the room in which the mice had been reared, whereas the novelty stress test was performed in a dark room that was separated from the breeding room. By combining the above conditions, the mice were divided into 12 groups.

Sample preparation

Mice were killed by decapitation immediately after the 20-min stress session. The brains were removed and, as quickly as possible, the prefrontal cortex and midbrain were dissected out on glass plates over ice. The samples were weighed and treated with 1000 μ l of an ice-cold 0.2 M perchloric acid (PCA) solution containing 0.2 mM sodium pyrosulfite, 0.01% EDTA-2Na, and 0.5 μ M isoproterenol (ISO) as an internal standard per 100 mg wet tissue. The solution was sonicated and then cen-

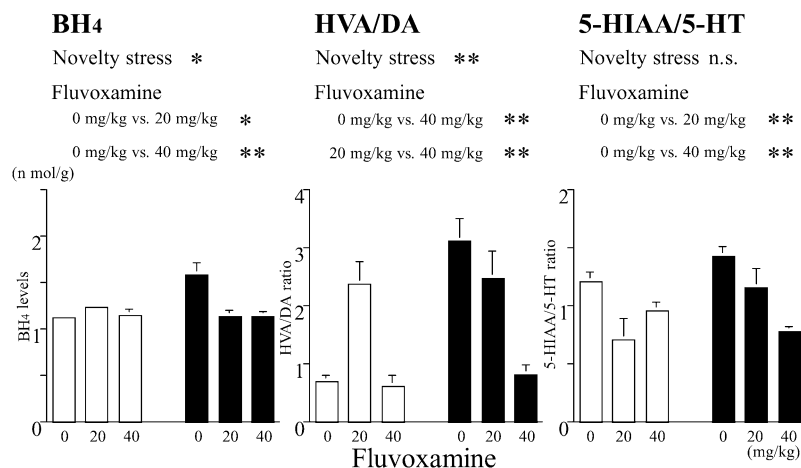
trifuged at 10,000 g for 20 min at 4°C. The supernatant was filtered through a Millipore HV filter (0.45 μ m pore size) and then subjected to both high-performance liquid chromatography (HPLC) with electrochemical detection (ECD) of monoamines (DA, 5-HT) and their metabolites (homovanilic acid, HVA; 5-hydroxyindoleacetic acid, 5-HIAA), and HPLC with fluorimetric detection (FD) of BH₄.

HPLC-ECD determination of brain levels of monoamines and their metabolites

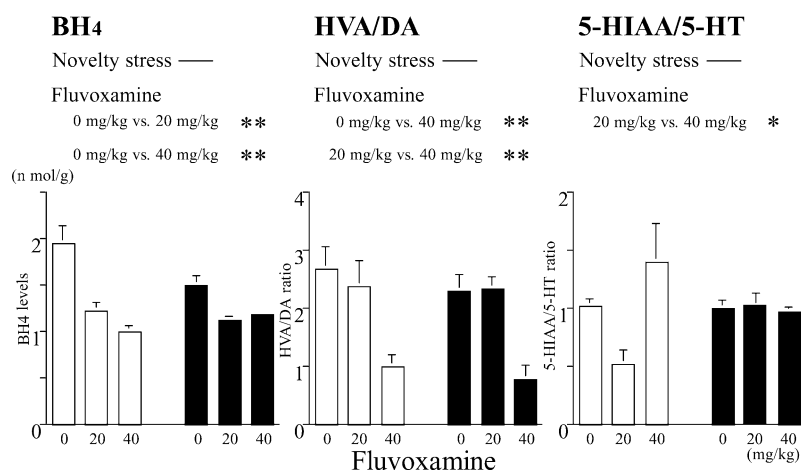
The levels of DA, HVA, 5-HT, and 5-HIAA in the brain extracts were measured by HPLC with ECD. The system employed for HPLC-ECD consisted of a CMA/200 autosampler (CMA/Microdialysis AB, Stockholm, Sweden), a micro LC pump (BAS, West Lafayette, Ind., USA), an LC-4C ECD (BAS), a Bio-Phase ODS-4 51-6034 column (4.0 \times 110 mm; BAS), a CR-6A recorder (Shimadzu, Kyoto, Japan), an LC-26A vacuum degasser (BAS),

Fig. 3 Changes in BH₄ levels, and HVA/DA and 5-HIAA/5-HT ratios in the midbrain elicited by novelty stress and by fluvoxamine. **A** group-housing condition (*n*=48); **B** isolation-housing condition (*n*=45). *White bars*, non-stress (*n*=46); *black bars*, novelty stress (*n*=47, *n*=93 total). Fluvoxamine: 0:0 mg/kg (*n*=29); 20:20 mg/kg (*n*=32); 40:40 mg/kg (*n*=32, *n*=93 total). Each bar indicates the final group division. The number of animals used for each group was eight, except in the case of the isolation-housed, stress, 0 mg/kg group (*n*=7), and isolation-housed, non-stress, 0 mg/kg group (*n*=6). Values are shown as the mean±SEM. *Asterisks* indicate the results of the Tukey-Kramer test for novelty stress and fluvoxamine in each housing and stress condition: **P*<0.05, ***P*<0.01, n.s. not significant. In the isolation-housing condition, the post hoc test for novelty stress was not performed because the MANOVA result was not significant

A Group housing



B Isolation housing



and a CTO-10A column heater set at 35°C (Shimadzu). The mobile-phase solution consisted of 0.1 M tartaric acid–0.1 M sodium acetate buffer, pH 3.2, containing 0.5 mM EDTA-2Na, 555 μM sodium 1-octane sulfonate, and 5% acetonitrile. The flow rate was 700 μl/min. The concentration of each compound was calculated by comparison with both the internal and the external standards.

HPLC-FD by post-column sodium nitrite oxidation for the determination of brain levels of BH₄

Tani and Ohno developed a method for the direct measurement of BH₄, the active form of bipterin (Tani and Ohno 1993), and we used this method to measure BH₄ levels in the present study. BH₄ (SIGMA) was stored in 0.1 M HCl (20 mM), and was prepared in 0.01 M HCl as an external standard (0.25 μM) immediately before sample injection. This system consisted of two LC-10AD pumps (Shimadzu), a CMA/200 autosampler, a Cosmosil 5C18

column (4.6×250 mm), a CR-6A recorder (Shimadzu), an LC-26A vacuum degasser, and a PF-10A FD (Shimadzu). The excitation wavelength was 350 nm, and the emission wavelength was 440 nm. The temperature of the reaction coil was set at 80°C using a column heater. The concentration of BH₄ was calculated by comparison with an external standard. The mobile phase was 0.1 M sodium phosphate buffer (pH 2.9) containing 5% methanol, 3 mM sodium 1-octane sulfonate, 0.1 mM EDTA-2Na, and 0.1 mM ascorbic acid (to prevent oxidation). The flow rate was 1.0 ml/min. Reduced pterins were oxidized by NaNO₂ (5 mM; flow rate: 1.0 ml/min) in the reaction coil (80°C).

Statistical analyses

To examine differences in the levels of BH₄, and in the ratios of HVA/DA and 5-HIAA/5-HT, three-way MANOVA (Wilks's lambda) for housing condition, novelty stress, and fluvoxamine was conducted on dependent measures in each brain region. Further analyses were performed to

consider the interactions. In each housing condition, i.e. group-housing and isolation-housing, two-way MANOVA (Wilks's lambda) for novelty stress and fluvoxamine was conducted on dependent measures in each brain region, followed by the Tukey-Kramer test. There were some missing values: in both regions, isolation-housing/stress/0 mg/kg ($n=7$, due to undetected DA in one animal), and in the midbrain, isolation-housing/non-stress/0 mg/kg ($n=6$, due to undetected DA in two animals).

Results

Prefrontal cortex

Three-way MANOVA (Wilks's lambda) for housing condition, novelty stress, and fluvoxamine was conducted for BH₄ levels, and to determine the HVA/DA and 5-HIAA/5-HT ratios. Housing condition [$F(3, 81)=3.630$, $P=0.0163$] and fluvoxamine [$F(6, 162)=12.013$, $P<0.0001$] significantly altered the dependent measures, whereas novelty stress [$F(3, 81)=1.663$, $P=0.1814$] did not. The interactions between housing condition and novelty stress [$F(3, 81)=4.932$, $P=0.0034$], housing condition and fluvoxamine [$F(6, 162)=9.153$, $P<0.0001$], and novelty stress and fluvoxamine [$F(6, 162)=4.527$, $P=0.0003$] were significant. The interaction among housing condition, novelty stress, and fluvoxamine [$F(6, 162)=2.749$, $P=0.0143$] was also significant.

In the group-housing condition, two-way MANOVA for novelty-stress and fluvoxamine was conducted on dependent measures. Novelty stress [$F(3, 40)=7.011$, $P=0.007$] and fluvoxamine [$F(6, 80)=4.722$, $P=0.0004$] significantly altered the dependent measures. The interaction between novelty stress and fluvoxamine was significant [$F(6, 80)=4.526$, $P=0.0005$]. The post hoc test revealed that novelty stress significantly increased BH₄ levels ($P<0.01$, Fig. 2A), and fluvoxamine significantly decreased the 5-HIAA/5-HT ratio (0 mg/kg versus 20 mg/kg, $P<0.05$; 0 mg/kg versus 40 mg/kg, $P<0.05$; Fig. 2A). In the isolation-housing condition, two-way MANOVA for novelty stress and fluvoxamine was conducted on dependent measures. Novelty stress [$F(3, 39)=1.363$, $P=0.2683$] did not alter these measures, whereas fluvoxamine [$F(6, 78)=11.442$, $P<0.0001$] significantly altered them. The interaction between novelty stress and fluvoxamine was significant [$F(6, 78)=3.419$, $P=0.0048$]. The post hoc test revealed that fluvoxamine significantly decreased BH₄ levels (0 mg/kg versus 20 mg/kg, $P<0.01$; 0 mg/kg versus 40 mg/kg, $P<0.01$; Fig. 2B) and HVA/DA ratio (0 mg/kg versus 20 mg/kg, $P<0.05$; 0 mg/kg versus 40 mg/kg, $P<0.01$; Fig. 2B).

Thus, in the group-housing condition, novelty stress was found to increase BH₄ levels and fluvoxamine to decrease 5-HT turnover. In the isolation-housing condition, novelty stress did not alter dependent measures and fluvoxamine decreased BH₄ levels and DA turnover.

Midbrain

Three-way MANOVA (Wilks's lambda) for housing condition, novelty stress, and fluvoxamine was conducted for BH₄ levels, and to determine the HVA/DA and 5-HIAA/5-HT ratios. Neither housing condition [$F(3, 79)=2.251$, $P=0.0889$] nor novelty stress [$F(3, 79)=1.646$, $P=0.1854$] altered dependent measures, whereas fluvoxamine [$F(6, 158)=22.222$, $P<0.0001$] significantly altered them. The interactions between housing condition and novelty stress [$F(3, 79)=4.513$, $P=0.0057$], housing condition and fluvoxamine [$F(6, 158)=4.790$, $P=0.0002$], and novelty stress and fluvoxamine [$F(6, 158)=4.470$, $P=0.0003$] were significant. The interaction among housing condition, novelty stress, and fluvoxamine [$F(6, 158)=4.807$, $P=0.0002$] was also significant.

In the group-housing condition, two-way MANOVA for novelty stress and fluvoxamine was conducted on dependent measures. Novelty stress [$F(3, 40)=5.011$, $P=0.0048$] and fluvoxamine [$F(6, 80)=9.868$, $P<0.0001$] significantly altered the dependent measures. The interaction between novelty stress and fluvoxamine was significant [$F(6, 80)=6.807$, $P<0.0001$]. The post hoc test revealed that novelty stress significantly increased BH₄ levels ($P<0.05$, Fig. 3A) and the HVA/DA ratio ($P<0.01$, Fig. 3A), and fluvoxamine significantly decreased BH₄ levels (0 mg/kg versus 20 mg/kg, $P<0.05$; 0 mg/kg versus 40 mg/kg, $P<0.01$; Fig. 3A), and decreased the HVA/DA (0 mg/kg versus 40 mg/kg, $P<0.01$; 20 mg/kg versus 40 mg/kg, $P<0.01$; Fig. 3A) and 5-HIAA/5-HT (0 mg/kg versus 20 mg/kg, $P<0.05$; 0 mg/kg versus 40 mg/kg, $P<0.05$; Fig. 3A) ratios. In the isolation-housing condition, two-way MANOVA for novelty stress and fluvoxamine was conducted on dependent measures. Novelty stress [$F(3, 37)=1.044$, $P=0.3845$] did not significantly alter the dependent measures, whereas fluvoxamine [$F(6, 74)=13.336$, $P<0.0001$] did significantly alter them. The interaction between novelty stress and fluvoxamine was significant [$F(6, 74)=3.264$, $P=0.0067$]. The post hoc test revealed that fluvoxamine significantly decreased BH₄ levels (0 mg/kg versus 20 mg/kg, $P<0.01$; 0 mg/kg versus 40 mg/kg, $P<0.01$; Fig. 3B), decreased the HVA/DA ratio (0 mg/kg versus 40 mg/kg, $P<0.01$; 20 mg/kg versus 40 mg/kg, $P<0.01$; Fig. 3B), and significantly increased 5-HIAA/5-HT ratio (20 mg/kg versus 40 mg/kg, $P<0.05$; Fig. 3B).

Thus, in the group-housing condition, novelty stress increased BH₄ levels and DA turnover, and fluvoxamine decreased BH₄ levels, as well as the DA and 5-HT turnover. In the isolation-housing condition, novelty stress did not alter dependent measures and fluvoxamine decreased BH₄ levels and DA turnover, whereas fluvoxamine increased 5-HT turnover.

Discussion

Human depression has a number of etiological risk factors. Both environmental and genetic factors have been

associated with the pathogenesis of the disease (Kendler et al. 1993). Among environmental factors, adverse life events such as interpersonal loss (e.g. separation) or an absence of social support (e.g. that occurring with a loss of social contact) appear to play important roles in the onset and relapse of depression (Paykel 1994). In the present study, we investigated the effects of fluvoxamine on BH₄ levels and on the DA and 5-HT turnover ratio in the mesoprefrontal system of mice using an animal model incorporating these two environmental risk factors. Isolation-housing is known to change the activities in the mesoprefrontal monoaminergic system. Isolation-housing suppresses TPH activity in the midbrain (Yanai and Sze 1983), whereas it increases the accumulation of the monoamine precursor, dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan (5-HTP) in the cerebral cortex (Miachon et al. 1993). Isolation has also been shown to increase the DA level and decrease the 5-HIAA/5-HT ratio in the prefrontal cortex (Jones et al. 1992), and to increase the KCl-induced release of 5-HT and DA from slices of prefrontal cortex (Jaffe 1998). Finally, Crepsi et al. (1992) showed by *in vivo* voltammetric analysis of the prefrontal cortex that isolation-housing prolonged 5-HT release and increased DA release following KCl or fenfluramine treatment.

In the prefrontal cortex and midbrain in the present study, novelty stress increased BH₄ levels under the group-housing condition (Figs 2A, 3A), whereas these levels were not altered under the isolation-housing condition (Figs 2B, 3B). Our recent study also suggested that novelty stress increases BH₄ levels in the hippocampus, although they did not change under the isolation-housing condition (Miura et al. 2004). According to these results, it appears likely that BH₄ plays a role in stress response mechanisms, and that isolation-housing attenuates these changes. A recent review demonstrated that intracellular concentrations of BH₄, which are mainly determined by GTP cyclohydrolase 1 (GCH), probably regulate the activity of TH, TPH, and also NOS (Nagatsu and Ichinose 1999). Thus, the increase in BH₄ levels elicited by novelty stress may have been related to the activities of TH, TPH, and NOS. Previous studies have shown elevations in brain TH and TPH activities elicited by physiological stress (Boadle-Biber et al. 1989; Serova et al. 1998; Chamas et al. 1999). Physiological stress has also been shown to increase NOS I mRNA levels in the hypothalamic paraventricular nucleus (Kishimoto et al. 1996). Thus, novelty stress may have elevated GCH activity, increased the BH₄ concentration, and differentially regulated TH, TPH, and NOS I activities in each brain region. Although the mechanisms of the BH₄ elevation elicited by novelty stress and the effect of isolation-housing on this type of elevation remain unknown, our results suggest that isolation-housing suppressed the elevation of BH₄ levels elicited by novelty stress. Further *in vivo* study using GCH inhibitors will help to clarify the mechanisms of these stress responses of BH₄.

In the midbrain, novelty stress enhanced DA turnover under the group-housing condition (Fig. 3A), whereas it

did not alter DA turnover under the isolation-housing condition (Fig. 3B). Novelty stress did not alter DA turnover in the prefrontal cortex (Fig. 2A,B). Thus, isolation-housing may have attenuated the increase in DA turnover elicited by novelty stress in the midbrain. Our previous study suggested that isolation-housing suppressed the elevation of monoamine (DA and 5-HT) turnover elicited by novelty stress (Miura et al. 2002a), although the regions in which such suppression was evident in that study, i.e. the prefrontal cortex and the nucleus accumbens (Miura et al. 2002a), differed from the regions of the present study. Although in our previous studies we reported that novelty stress significantly changed the levels of monoamines and their metabolites (Miura et al. 2002a,b), novelty stress was not found to significantly alter either of these levels in the present study. These differences in the stress response between our present and previous studies may be attributable, at least in part, to differences in the stress-session procedures. Here, we employed a non-stress condition in which animals were habituated twice to the novel environment before final exposure, and a stress condition in which they experienced the novel environment without prior habituation. In our previous studies, animals in the non-stress group were killed without exposure to the novel environment. We cannot rule out the possibility that these habituation sessions influenced CNS activity in the non-stress group. The differences between housing cages may also have had an influence. In the previous studies we used hanging-type cages with wire-mesh bottoms to minimize the influence of handling and social experience (Holson et al. 1991; Krebs-Thomson et al. 2001). However, the use of these cages may itself have constituted a chronic stressor. Finally, the difference in species used (rats versus mice) may also have contributed to the difference in results. Despite these differences, however, isolation-housing was clearly shown to attenuate the elevation of DA turnover elicited by novelty stress.

Fluvoxamine was also shown to suppress BH₄ levels in this study (Figs 2B, 3A,B), with the exception of the group-housing condition in the prefrontal cortex (Fig. 2A). In both regions, two-way MANOVA revealed significant interactions between novelty stress and fluvoxamine in each housing condition; thus fluvoxamine attenuated the elevation of BH₄ levels elicited by novelty stress. Our recent study demonstrated that fluvoxamine decreased BH₄ levels in the hippocampus of mice (Miura et al. 2004). Because chronic antidepressant treatment has been shown to suppress TH (Nestler et al. 1990) as well as TPH activity (Lapierre et al. 1983), the decrease in BH₄ levels elicited by fluvoxamine would seem to have attenuated the activities of these enzymes. Recently, NOS inhibitors have been shown to exhibit antidepressant-like effects in animal models (Harkin et al. 1999; Karolewicz et al. 1999; Da Silva et al. 2000). Paroxetine, a selective serotonin reuptake inhibitor (SSRI), is known to act as an NOS inhibitor (Finkel et al. 1996). Thus, antidepressants may possess clinical potency by inhibiting NOS activities. Fluvoxamine, an inhibitor of cytochrome P450 isozymes

that are structurally homologous to NOS (Richelson 1997), may influence NOS activity by decreasing BH₄ levels, although the mechanism of BH₄ suppression remains unknown. However, further research into the relation between alterations in BH₄ levels and GCH activity, and that between changes in BH₄ levels and NOS I activity elicited by fluvoxamine will help to clarify the role of NOS I in the clinical efficacy of the drug.

Fluvoxamine was found to inhibit DA turnover (Figs 2B, 3A,B), with the exception that there was no change in DA turnovers in the prefrontal cortex under the group-housing condition (Fig. 2A). In both regions, two-way MANOVA revealed significant interactions between novelty stress and fluvoxamine in each housing condition, and thus fluvoxamine attenuated the elevation of DA turnover elicited by novelty stress. To our knowledge, this is the first study showing a decrease in BH₄ levels and a simultaneous inhibition of DA turnover elicited by fluvoxamine. The mechanism of the effects of fluvoxamine on the DA system remains unknown. We suspect that the inhibition of 5-HT transporter (SERT) activity elicited by fluvoxamine cannot solely account for the inhibition of DA turnover. It is likely that other pharmacological effects related to the changes in BH₄ levels also played a role in inducing these changes. We propose here two possible explanations for these findings, although these are only speculation at present. The first explanation involves the regulation of DA neuron activity by the innervation of 5-HT neurons. The 5-HT innervations of the DA system are thought to attenuate the activity of DA neurons, and thus fluvoxamine may have potentiated the attenuation by increasing 5-HT levels (Di Mascio et al. 1998; Dong et al. 1999). The second possibility is that fluvoxamine may have suppressed TH activity via the decrease in BH₄ levels, and thereby suppressed DA biosynthesis. A study using 6-pyruvoyltetrahydropterin synthase-knockout mice (i.e. mice in which the second step of BH₄ biosynthesis is blocked) showed that the suppression of TH and NOS activities in the brain did not affect TPH activity (Sumi-Ichinose et al. 2001). In a study by Flatmark (2000), TH activity was highly dependent on the intracellular concentration of BH₄.

Fluvoxamine decreased the 5-HIAA/5-HT ratio under the group-housing condition in both regions (Figs 2A, 3A). Under the isolation-housing condition fluvoxamine did not alter the 5-HIAA/5-HT ratio in the prefrontal cortex (Fig. 2B), whereas it increased this ratio in the midbrain (Fig. 3B).

Although the original monoamine hypothesis has advanced our understanding of the etiology and pathophysiology of human depression, it does not address several major issues. The hypothesis has evolved to include adaptive changes in receptors to explain why there should be only a gradual clinical response to antidepressant treatment when the increase in availability of monoamine is rapid (Hirschfeld 2000). On the other hand, the dysfunction of SERT is the target of some of the newest forms of antidepressant pharmacotherapy, including the SSRIs (Leonard 2000). Activity of the SERT in

platelets is reduced in patients with depression (Owens and Nemeroff 1994), and changes in the SERT in platelets have been found to correlate with response to treatment (Leonard 2000). A study using single photon emission computed tomography with [¹²³I]-2β-carbomethoxy-3β-(4-iodophenyl) tropane, the radiolabeled tracer binding with high affinity to SERT in the midbrain, revealed a reduction in the activity of the transporter in patients with depression (Malison et al. 1998). In the present study, fluvoxamine may have modified the activity and/or expression of SERT. The discrepancy of fluvoxamine-induced changes in 5-HT turnover between isolation and group-housing might be attributable to the difference in the responses of SERT activities to fluvoxamine between the two housing conditions, although the underlying mechanism remains to be clarified. Further, impaired activity of enzymes essential for monoamine synthesis may play a role in depression, although reports on this subject are few (Leonard 2000). Our results suggest the possibility that the decreased BH₄ levels elicited by fluvoxamine suppressed TH and/or TPH activity.

In the present study, both novelty stress and fluvoxamine induced changes in BH₄ levels, DA turnover, and 5-HT turnover in the mesoprefrontal system. In the group-housing animals, novelty stress significantly increased BH₄ levels. The suppression of BH₄ levels by fluvoxamine may have in turn been related to the suppression of DA turnover. As mentioned above, our results suggest the possibility that the clinical efficacy of fluvoxamine may be due to its influence on BH₄ levels as well as due to its effect on SERT. Further investigation of these potential mechanisms will help clarify the pathophysiology and pathogenesis of human depression.

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