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Synergetic Effects of Quetiapine and Venlafaxine in Preventing the Chronic Restraint Stress-Induced Decrease in Cell Proliferation and BDNF Expression in Rat Hippocampus

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ABSTRACT: Clinical studies show better response rates of patients with depression and schizophrenia to combinations of atypical antipsychotics and antidepressants, compared to responses to either type of drugs alone. Animal studies demonstrate that some antipsychotics and antidepressants increase neurogenesis and BDNF expression in the hippocampus, which is reduced in volume in patients with depression or schizophrenia. We hypothesized that the better therapeutic effects of combined treatment seen in schizophrenia and depression patients are related to the additive or synergistic effects of combined treatment on hippocampal neurogenesis and BDNF expression. To test this hypothesis, we investigated the effects of chronic administration of quetiapine, venlafaxine, and their combination, on hippocampal cell proliferation and BDNF expression in rats, when subjected to chronic restraint stress (CRS) during the last 2 weeks of a 3-week drug administration period. We found (1) CRS decreased hippocampal cell proliferation and BDNF expression; (2) chronic administration of quetiapine or venlafaxine dose-dependently prevented these decreases in hippocampal cell proliferation and BDNF expression caused by CRS (6 h/day for 14 days); (3) the combination of lower doses of quetiapine (5 mg/kg) and venlafaxine (2.5 mg/kg) increased hippocampal cell proliferation and prevented BDNF decrease in stressed rats, whereas each of the drugs exerted mild or no effects; (4) individual higher doses of quetiapine (10 mg/kg) or venlafaxine (5 mg/kg) exerted effects comparable to those produced by their combination. These results support our hypothesis and can lead to future studies to develop new therapeutic approaches for treatment-resistant depression and the negative symptoms of schizophrenia. © 2006 Wiley-Liss, Inc.

KEY WORDS: antidepressants; antipsychotics; hippocampus; cell proliferation; BDNF

INTRODUCTION

There are clinical reports that combinations of atypical antipsychotic drugs (APDs) and antidepressants are more effective for treatment-resistant depression patients and in treating the negative symptoms of schizophrenia. For example, the atypical APD olanzapine or risperidone enhanced the therapeutic response of depression to fluvoxamine (a selective serotonin reuptake inhibitor) (Hirose and Ashby, 2002). In another report, fluvoxamine improved negative symptoms when added to ongoing antipsychotic treatment (Silver and Nassar, 1992). Similarly, fluoxetine, another SSRI, improved negative symptoms in patients with schizophrenia (Goff et al., 1995).

In animal studies, chronic administration of antidepressants have been shown to increase neurogenesis (Malberg et al., 2000; Kodama et al., 2004) and brain-derived neurotrophic factor (BDNF) expression (Nibuya et al., 1995; Xu et al., 2003) in the hippocampus, a brain region that plays critical roles in learning and memory, and which is compromised in patients with depression (Sheline et al., 1996) and schizophrenia (Weiss et al., 2005). Atypical APDs also stimulate neurogenesis in adult rat brain (Wakade et al., 2002; Kodama et al., 2004) and upregulate BDNF expression in the hippocampus (Bai et al., 2003; Fumagalli et al., 2003).

BDNF is widely distributed in the brain, and synthesized predominantly in neurons; its expression is highest in the hippocampus and in the cerebral cortex (Ernfors et al., 1990; Hofer et al., 1990; Wetmore et al., 1990). In addition to providing neurotrophic support for cholinergic neurons, BDNF has been proposed to have a potential role in promoting the function and survival of dopaminergic, GABAergic, noradrenergic, and serotonergic neurons (for review, see Connor and Dragunow, 1998). The expression of both the BDNF gene and its corresponding protein has been shown to be regulated by a number of processes, such as seizures, restraint stress, neurotransmitter actions, and second messenger cascades, including cAMP (Humpel et al., 1993; Wetmore et al., 1994; Lindefors et al., 1995; Nibuya et al., 1995, 1996; Smith et al., 1995; Vaidya et al., 1997; Bain et al., 2004; Xu et al., 2004).

Neurogenesis is a process of generating functionally integrated neurons from progenitor cells (Ming and Song, 2005). In most mammals, active neurogenesis occurs throughout life in the subventricular zone of the lateral ventricles and in the subgranular zone

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(SGZ) of the dentate gyrus (DG). Newborn neuronal cells in the SGZ will migrate to the granular cell layer of the DG, integrate into the existing hippocampal circuitry (Cameron and McKay, 2001; van Praag et al., 2002), and play important roles in learning and memory formation (Gould et al., 1999; Shors et al., 2001; Shors, 2004).

On the basis of the aforementioned studies, we hypothesized that the better therapeutic effects seen in patients with schizophrenia or depression of a combination of antipsychotics and antidepressants (as compared to either type alone) are related to their additive or synergistic effects on hippocampal neurogenesis and BDNF expression. To test this hypothesis, we first reproduced a state of decreased hippocampal neurogenesis and BDNF expression in an animal model of chronic restraint stress (CRS), and then examined the effects of various doses of quetiapine and venlafaxine, or their combinations, on hippocampal cell proliferation and BDNF expression in these stressed rats.

Quetiapine is an atypical antipsychotic; these drugs not only have affinity for D_2 receptors, but also affect other receptors including other dopamines (D_1, D_3, D_4), serotonins (5-HT_{2A}, 5-HT_{1A}, and 5-$HT_{3,6}$ receptors), and α-1 adrenergic receptors (Richelson, 1999; Tatsumi et al., 1999; Miyamoto et al., 2005). Quetiapine is effective for both the positive and negative symptoms of schizophrenia (Borison et al., 1996; Arvanitis and Miller, 1997; Small et al., 1997). In patients with schizophrenia, quetiapine also has beneficial effects on cognitive function (Sax et al., 1998) and relieves depressive symptoms (Purdon et al., 2001; Sajatovic et al., 2002). In animal studies, quetiapine attenuates the reduction of hippocampal BDNF expression induced by CRS (Xu et al., 2002), or the NMDA receptor antagonist MK-801 (Fumagalli et al., 2004), and it reverses the CRS-induced suppression of hippocampal neurogenesis (Luo et al., 2005).

Venlafaxine is a dual serotonin (5-HT)/norepinephrine (NE) reuptake inhibitor. At lower doses, it functions as an SSRI (Stahl, 1998). At medium to high doses (>150 mg/day), venlafaxine is useful for melancholic, severely depressed patients and for those refractory to other antidepressants (Gutierrez et al., 2003). Chronic venlafaxine treatment increases serum BDNF levels in patients with major depressive disorder (Aydemir et al., 2005) and upregulates BDNF expression in rat hippocampus (Xu et al., 2003). In addition, chronic administration of venlafaxine stimulates hippocampal cell proliferation (Khawaja et al., 2004) and reverses the decrease in hippocampal cell proliferation and BDNF expression caused by CRS (Xu et al., 2004).

There are clinical reports on the use of the combination of quetiapine and venlafaxine in patients with psychiatric disorders. For example, Madhusoodanan et al. (2000) reported that quetiapine markedly decreased positive and negative symptoms of elderly patients with psychotic disorders. These patients were treated previously with conventional antipsychotics or other atypical antipsychotics, but had no satisfactory response. After quetiapine treatment, preexisting extrapyramidal symptoms in some patients were diminished. In addition, no adverse consequences occurred when lithium, carbamazepine, valproic acid, or venlafaxine were given concurrently. In a more recent clinical study, the combination of quetiapine and venlafaxine was successfully used for patients with treatment-refractory obsessive-compulsive disorder (Denys et al., 2002).

MATERIALS AND METHODS

Materials

The sources for the reagents and supplies used are as follows: Quetiapine (AstraZeneca Canada, Mississauga, Ontario); venlafaxine (Wyeth-Ayerst Research, Philadelphia, NJ); 5-bromo-20-deoxyuridine (BrdU) and 3,3′-diaminobenzidine (DAB) (Sigma, St. Louis, MO); the primary antibody to BDNF (RDI, Flanders, NJ), which was raised in rabbits, is specific for BDNF with the epitope mapping at the amino terminus of the mature form of human BDNF (identical to the corresponding mouse sequence) and is nonreactive with NGF, NT-3, or NT-4; the primary anti-mouse antibody to BrdU (Zymed Laboratories Inc, South San Francisco, CA); the ABC reagent kit for immunhistochemistry (Vector Laboratories, Burlingame, CA); the BCA protein assay reagent kit (Pierce, Rockford, IL); Horseradish peroxidase-linked secondary antibodies and polyvinyldifluoride membranes (Biorad, Hercules, CA); the chemoluminescent Western blot kit (Amersham, Piscataway, NJ); Kodak Biomax films (Eastman Kodak, Rochester, NY). Adult male Sprague-Dawley rats weighing 200–225 g were purchased from Charles River Laboratories (St. Constant, QC). They were group housed and maintained on a 12:12 h light/dark cycle with food and water freely available. Quetiapine and venlafaxine were dissolved in saline containing 0.8% acetic acid.

Experimental Design and Animal Groups

All of the procedures involving animals were in accordance with the guidelines established by the Canadian Council on Animal Care, and approved by the University of Saskatchewan Animal Care Committee.

In the first experiment, there were nine groups and each group had five rats. Animals in the nonstress (NS) group received neither CRS nor drug injection. In the stress (Str) group, the rats received CRS (6 h/day for 14 days) in plexiglas tubes (Xu et al., 2002). In the other seven groups, rats were intraperitoneally (i.p.) injected with the vehicle (0.8% acetic acid in saline), quetiapine (5 or 10 mg/kg), venlafaxine (2.5 or 5 mg/kg), 5 mg/kg quetiapine plus 2.5 mg/kg venlafaxine, or 10 mg/kg quetiapine plus 5 mg/kg venlafaxine once a day for 21 days, and during days 8–21 the rats were subjected to the same CRS as those in the group Str. These groups were designated as Veh, Q_5, Q_{10}, $V_{2.5}$, V_5, $Q_5 + V_{2.5}$, or $Q_{10} + V_5$, respectively. The doses of quetiapine and venlafaxine used in the present study were chosen based on previous studies (Xu et al., 2002, 2003; Fumagalli et al., 2004; Chen et al., 2005; Luo et al., 2005). Two hours after the last session of CRS (or at the same time for NS group), rats from each group were given

(i.p.) BrdU (100 mg/kg) dissolved in saline. After 24 h, the rats were sacrificed under deep anesthesia with chloral hydrate (400 mg/kg, i.p.) and their brains were processed according to immunohistochemical protocols, to detect the newborn cells that had incorporated BrdU during division and to measure BDNF immunoreactivity in the hippocampus (described later). We chose animals for injections (vehicle, drugs, BrdU, and chloral hydrate) by choosing the first rat (to be injected) from a randomly selected group that had the most animals waiting; the order of first injected rats of each group was recorded and followed by next injections until injections were finished on that day. In this way, the time period between BrdU injection and sacrifice of rats was controlled and compared across various groups.

In the second experiment, there were only six groups (NS, Str, Veh, Q_5, $V_{2.5}$, and $Q_5 + V_{2.5}$), and each group had five rats. Except for BrdU injection, these animals received the same treatments as those in the same groups of the first experiment. After decapitation under deep anesthesia with chloral hydrate (400 mg/kg, i.p.), the hippocampal tissue of these rats was processed for Western blot analysis (described later).

Immunohistochemistry

Twenty-four hours after BrdU injection, the rats were deeply anesthetized with chloral hydrate (400 mg/kg, i.p.) and perfused with 250 ml of 0.1 M phosphate buffered saline (PBS, pH 7.4), followed by 300 ml of 4% paraformaldehyde in PBS. Their brains were removed, postfixed in the same fixative for 1 day, and then cryoprotected in 20% sucrose for 2–3 days at 4°C. Brain tissue blocks were frozen in 2-methylbutane prechilled with dry ice and then stored at −70°C until use. Serial coronal sections (30 μm) of the brains were cut through the hippocampus on a freezing microtome and collected in 6-well plates containing 0.01 M PBS.

For immunohistochemical staining of BDNF, free-floating sections were pretreated with 0.6% hydrogen peroxide in PBS for 30 min, washed with PBS, and incubated for 1 h at room temperature (22°C) with a blocking solution composed of 0.3% Triton X-100 and 5% normal goat serum in PBS. Sections were then incubated with the primary antibody to BDNF (1:1,000) in the blocking solution at 4°C overnight, followed by an additional 2 h at 22°C. After rinsing in PBS, the sections were incubated in biotinylated rabbit secondary antisera for 2 h at 22°C. Following PBS rinses, the sections were incubated in avidin-biotin-horseradish peroxidase for 1 h at 22°C. Finally, the sections were developed with a solution of 0.03% DAB and 0.03% hydrogen peroxide in Tris/HCl-buffered saline (TBS, 0.05 M, pH 7.6).

For the detection of BrdU, the sections were incubated in 50% formamide/2× SSC at 65°C for 2 h, followed by PBS rinses. The sections were then incubated in 2 N HCl for 30 min and in boric acid for 10 min. After the PBS rinses, the sections were subjected to the same procedure as described earlier for BDNF. The primary antibody to BrdU was used at a dilution of 1:1,000. The immunohistochemical controls for the detection of BDNF and BrdU were performed as described earlier, except for the omission of the primary antibodies. All controls were negative.

Densitometric Measurement of BDNF Immunohistochemical Staining and Quantification of BrdU-labeled Cells in the Hippocampus

Every sixth brain sections in the range of Bregma −2.3 to Bregma −5.8 (Paxinos and Watson, 1986) were observed and their digital images were acquired using an Olympus BH2-RFCA microscope fitted with a Spot-RT digital camera (Diagnostic Instruments, Sterling Heights, MI) and analyzed using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). For BDNF immunohistochemical staining, the measured targets were CA1 and CA3 of the pyramidal cell layer of Ammon's horn and the granule cell layer of DG. The areas bordering both sides of the targets were also measured and used as background. The differences between the gray scale values of the targets and the values of the backgrounds were calculated for each section and designated as OD. BrdU-labeled cells located in the hilus and SGZ of DG were counted under 400× or 1,000× magnification in cases of cell clusters. Fifteen or sixteen sections from each animal were used for the counting. The number of BrdU-labeled cells per section was determined and multiplied by six to obtain the number of BrdU-labeled cells per DG. This protocol has been used in previous studies reporting BrdU-labeled cells (Nakagawa et al., 2002; Xu et al., 2004). The number of BrdU-labeled cells in DG has been thought to be an index of hippocampal neurogenesis, as the majority of BrdU-labeled cells in this brain area express neuronal markers, such as NeuN, Calbindin-D_{28k}, NF-200, or MAP-2, but not GFAP (Jiang et al., 2003; Malberg and Duman, 2003). Furthermore, in a recent study of stressed rats, we found parallel changes between the numbers of BrdU-labeled cells and pCREB-positive cells in SGZ (Luo et al., 2005). Previous studies have demonstrated that almost all immature neurons expressing PSA-NCAM show positive pCREB immunoreactivity in SGZ (Nakagawa et al., 2002; Park et al., 2004). Therefore, in the present study, we did not perform double- or triple-labeling to identify the phenotype of BrdU-labeled cells.

Western Blot Analysis

The levels of BDNF protein in the dissected hippocampi were measured by Western blot analysis. Each frozen hippocampus was homogenized and lysed in 2 ml of PBS containing 1% Nonidet P-40 and the following protease inhibitors: phenylmethylsulfonyl fluoride (100 μg/ml), leupeptin (1 μg/ml), pepstatin (1 μg/ml), and aprotinin (1 μg/ml). Lysates were cleared by centrifugation, and the total protein concentration in each sample was determined spectrophotometrically at A^{595} nm using the BCA protein assay reagent kit. Samples containing 50 μg protein per 10 μl were denatured in gel-loading buffer and separated on 12% SDS-PAGE gels. Following electrophoresis, the proteins were transferred to polyvinyldifluoride membranes. After preblocking in 5% skimmed milk powder in PBS, the membranes were incubated at 4°C with the primary antibody to BDNF

TABLE 1.

Chronic Restraint Stress Decreased BDNF Immunoreactivity in the Hippocampus

	CA1	CA3	DC
NS	100 ± 13.8	100 ± 10.7	100 ± 7.1
Str	52 ± 4.2**	56 ± 4.4**	41 ± 24**

Data are expressed as mean ± SE.
**$P < 0.01$, Str vs. NS.

(1:1,000) for 72 h, and then incubated for 2 h at 22°C with horseradish peroxidase-linked secondary antibodies. The control for protein loading was performed by reprobing membranes with an antibody against β-actin (Santa Cruz Biotechnology, Santa Cruz, CA). Immunoreactive bands were detected by a chemoluminescent Western blot kit, and then exposed to Kodak Biomax film. Images were collected with the ImageMaster VDS (Amersham Pharmacia Biotech, Piscataway, NJ) and analyzed using Image-Pro Plus (Media Cybernetics, Silver Spring, MD). The optical density value of each given band was corrected based on the corresponding β-actin band.

Statistical Analysis

Student's *t*-test was used to compare the results of the groups NS and Str. One-way analysis of variance (ANOVA) was performed on the data of the groups Veh, Q_5, and Q_{10}, and the groups Veh, $V_{2.5}$, and V_5. Two-way ANOVA was performed on the data of the groups Veh, Q_5, $V_{2.5}$, and $Q_5 + V_{2.5}$, and the groups Veh, Q_{10}, V_5, and $Q_{10} + V_5$, followed by Newman-Keuls post hoc comparisons.

RESULTS

CRS Decreased Hippocampal Cell Proliferation and BDNF Expression

The BrdU-labeled cells in the subgranular zone and the hilus of DG of rats were quantified. Student's *t*-test was performed for the animal groups NS and Str. The group Str had a lower number of BrdU-labeled cells, as compared to the group NS (2,996 ± 232 vs. 5,688 ± 288, $P = 0.003$).

Only the hippocampal pyramidal cell layer and the dentate granule cell layer showed intensive BDNF immunohistochemical staining in hippocampal sections (not shown). This feature allowed us to measure the intensities of BDNF immunohistochemical staining in the hippocampal subregions CA1, CA3, and DG; the results are shown in Table 1.

Chronic Administration of Quetiapine Blocked the CRS-induced Decrease in Hippocampal Cell Proliferation and BDNF Expression in a Dose-dependent Manner

The reduction in hippocampal cell proliferation caused by CRS was blocked by chronic administration of 5 mg/kg or 10 mg/kg quetiapine (Fig. 1A). One-way ANOVA showed that the treatment produced an evident effect ($F_{(2,\ 12)} = 16.78$, $P = 0.0003$). Multiple post hoc comparisons of the results showed that Q_5 and Q_{10} had significantly more BrdU-labeled cells as compared to Veh.

Only the higher dose (10 mg/kg) of quetiapine blocked the stress-induced decrease in the intensities of BDNF immunohistochemical staining in all three subregions of the hippocampus. Although 5 mg/kg quetiapine showed a mild increase relative to Veh, the effect was not significant (Fig. 1B).

Chronic Administration of Venlafaxine Blocked the CRS-induced Decrease in Hippocampal Cell Proliferation and BDNF Expression in a Dose-dependent Manner

Only the higher dose (5 mg/kg) of venlafaxine blocked the stress-induced reduction in hippocampal cell proliferation (Fig. 2A), although one-way ANOVA revealed a significant effect of the chronic administration of venlafaxine ($F_{(2,\ 12)} = 30.64$, $P < 0.0001$). Similarly, only V_5 showed the higher intensities of BDNF immunohistochemical staining in all three subregions of the hippocampus as compared to Veh (Fig. 2B).

The Synergistic and Additive Effects of Quetiapine and Venlafaxine in Blocking the CRS-induced Decrease in Hippocampal Cell Proliferation and BDNF Expression

Two-way ANOVA on the numbers of BrdU-labeled cells in the groups Veh, Q_5, $V_{2.5}$, and $Q_5 + V_{2.5}$ revealed a significant

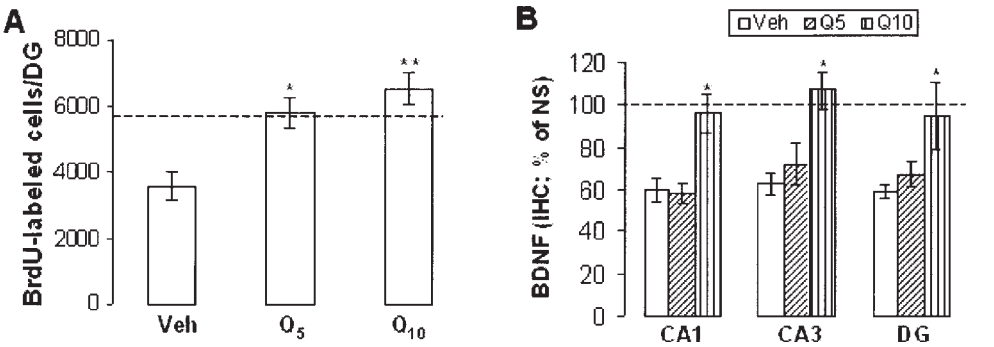

FIGURE 1. Chronic administration of quetiapine blocked the decrease in hippocampal cell proliferation (A) and BDNF immunoreactivity (B) caused by CRS. Data were expressed as mean ± standard error of mean (SEM). The dotted lines in A and B indicate the levels of NS group. *$P < 0.05$; **$P < 0.01$, compared to Veh.

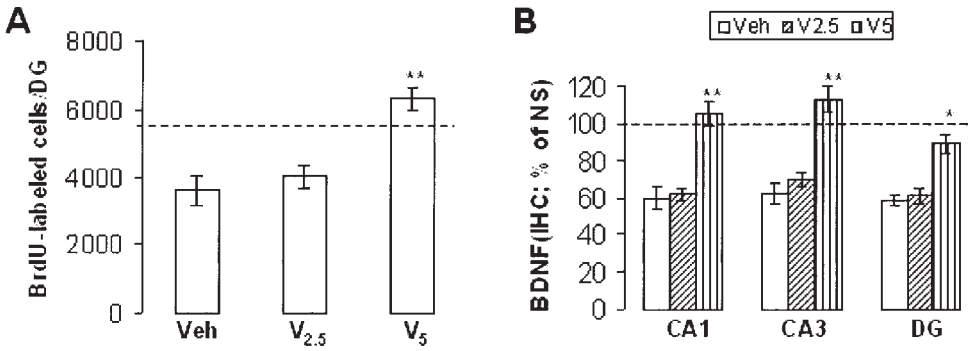

FIGURE 2. Chronic administration of venlafaxine blocked the decrease in hippocampal cell proliferation (A) and BDNF immunoreactivity (B) caused by CRS. Data were expressed as mean ± SEM. The dotted lines in A and B indicate the levels of NS group. *$P < 0.05$; **$P < 0.01$, compared to Veh.

interaction ($F_{(1, 16)} = 14.48$, $P = 0.0016$) between 5 mg/kg quetiapine and 2.5 mg/kg venlafaxine. Multiple post hoc comparisons showed that the groups Q_5 and $Q_5 + V_{2.5}$ had many more BrdU-labeled cells than the group Veh, whereas the $V_{2.5}$ group had a number comparable to that of Veh. When compared to the NS group, the values of the Q_5 group were comparable whereas the $Q_5+V_{2.5}$ group was still higher (Fig. 3A). The numbers of BrdU-labeled cells in the groups Veh, Q_5, $V_{2.5}$, $Q_5 + V_{2.5}$, and the differences (Δ-values) between Veh and each of the groups Q_5, $V_{2.5}$, $Q_5 + V_{2.5}$ are summarized in Table 2. The Δ-value of the groups $Q_5 + V_{2.5}$ and Veh is 7,198, whereas the sum of Δ-values of the groups Q_5, $V_{2.5}$, and Veh is only 2,626, suggesting a synergetic effect of the lower doses of quetiapine and venlafaxine in regulating hippocampal cell proliferation in the stressed rats. When we analyzed the data of the groups Veh, Q_{10}, V_5, $Q_{10} + V_5$ (Fig. 3B), no significant interaction between 10 mg/kg quetiapine and 5 mg/kg venlafaxine was found ($F_{(1, 16)} = 0.85$, $P = 0.37$), although each of Q_{10}, V_5, and $Q_{10} + V_5$ blocked the stress-induced suppression of hippocampal cell proliferation. The numbers of BrdU-labeled cells in the groups Veh, Q_{10}, V_5, $Q_{10} + V_{25}$, and the differences (Δ-values) between Veh and each of the groups Q_{10}, V_5, $Q_{10} + V_5$ are summarized in Table 3. The Δ-value of the groups $Q_{10} + V_5$ and Veh is 5,548, and the sum of Δ-values of the groups Q_{10}, V_5, and Veh is 5,636, suggesting an additive effect of the higher doses of quetiapine and venlafaxine in regulating hippocampal cell proliferation in the stressed rats.

Analysis on the OD values of BDNF immunohistochemical staining of the groups Veh, Q_5, $V_{2.5}$, $Q_5 + V_{2.5}$ showed that only the group $Q_5 + V_{2.5}$ had significantly higher values than the group Veh (Fig. 3C). For the groups Veh, Q_{10}, V_5, $Q_{10} + V_5$, all three drug-treatments showed the same effect of blocking BDNF immunoreactivity decrease caused by CRS (Fig. 3D).

Western blot analysis also showed that the group $Q_5 + V_{2.5}$ had a BDNF level comparable to that of NS, but higher than levels of the groups Veh, Q_5, $V_{2.5}$ (Fig. 4). This result is in accordance with the observations in hippocampal sections stained immunohistochemically and supports the notion that the lower doses of quetiapine and venlafaxine synergistically regulate BDNF expression in the hippocampus of stressed rats.

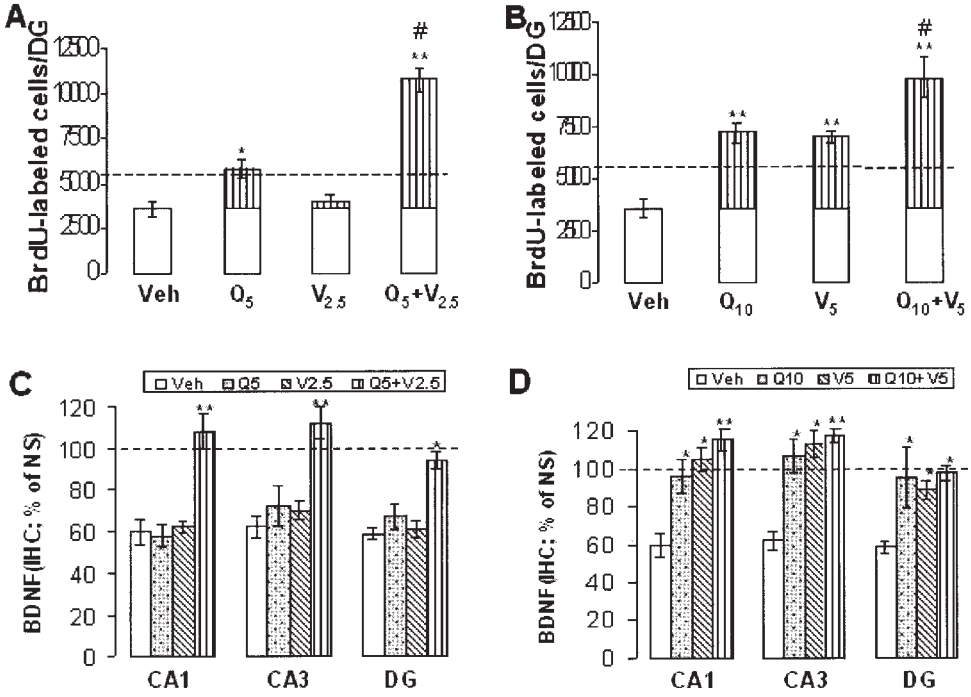

FIGURE 3. The synergetic and additive effects of quetiapine and venlafaxine in regulating hippocampal cell proliferation and BDNF expression of stressed rats. In A and B, the striped part of the groups Q_5, $V_{2.5}$, $Q_5 + V_{2.5}$, Q_{10}, V_5, and $Q_{10} + V_5$ indicates overages of BrdU-labeled cells in these groups when compared to the group Veh. The dotted lines indicate the levels of NS group. The data were expressed as mean ± SEM. *$P < 0.05$; **$P < 0.01$, compared to Veh. #$P < 0.05$, compared to NS.

TABLE 2.

Synergetic Effect of Q_5 and $V_{2.5}$ in Blocking the Stress-Induced Decrease in Hippocampal Cell Proliferation

NS (A)[a]	5,688 ± 283
Veh (B)[a]	3,596 ± 425
Q_5 (C)[a]	5,804 ± 457
$V_{2.5}$ (D)[a]	4,014 ± 341
$Q_5 + V_{2.5}$ (E)[a]	10,794 ± 673
(C-B) + (D-B)	2,626
E-B	7,198

[a]Values are given in mean ± SE.

DISCUSSION

CRS suppressed hippocampal cell proliferation and decreased BDNF expression in the hippocampus. The parallel decreases suggest that reduced BDNF levels may contribute to the suppression of cell proliferation in this paradigm. In support of this idea, it has been found that infusion of BDNF into the lateral ventricle of the adult rat leads to new neurons in the parenchyma of the striatum, septum, thalamus, and hypothalamus (Pencea et al., 2001); BDNF administrated to the DG leads to increased neurogenesis of granule cells (Scharfman et al., 2005). In addition to lower BDNF, elevated circulating levels of adrenal steroids may be another contributor to the stress-induced decrease in hippocampal cell proliferation as shown in previous studies that experimental increases in the levels of adrenal steroids result in significant decreases in the rate of granule cell production (Gould et al., 1991; Cameron and Gould, 1994), and that the decrease in the number of BrdU-labeled cells in the DG after fox odor exposure was eliminated by prevention of a stress-induced rise in adrenal hormone levels (Tanapat et al., 2001). In fact, lower BDNF may actually occur as a result of elevated adrenal steroids (Scaccianoce et al., 2003). In our experimental paradigm, elevated adrenal steroids and lower BDNF are possibly in the same causal pathway leading to decrease in neurogenesis.

TABLE 3.

Additive Effect of Q_{10} and V_5 in Blocking the Stress-Induced Decrease in Hippocampal Cell Proliferation

NS (A)[a]	5,688 ± 283
Veh (B)[a]	3,596 ± 425
Q_{10} (C)[a]	6,520 ± 485
V_5 (D)[a]	6,308 ± 302
$Q_{10} + V_5$ (E)[a]	9,144 ± 944
(C-B) + (D-B)	5,636
E-B	5,548

[a]Values are given in mean ± SE.

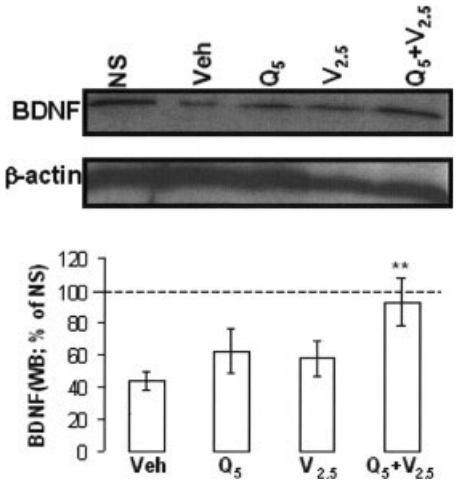

FIGURE 4. The synergetic effect of the lower doses of quetiapine and venlafaxine in blocking the stress-induced decrease in hippocampal levels of BDNF. The upper panel is a representative immunoblot made from hippocampal tissues of rats of the groups NS, Veh, Q_5, $V_{2.5}$, and $Q_5 + V_{2.5}$ as described in Materials and Methods. The bottom panel is a histogram showing the quantification of the immunochemically reactive bands in the Western blots. The dotted lines indicate the levels of NS group. The data were normalized by taking the value of NS group as 100%, and expressed as mean ± standard deviation (SD). $^{**}P < 0.01$, compared to the group Veh.

Both 5 and 10 mg/kg quetiapine effectively blocked the suppression of hippocampal cell proliferation caused by CRS (Fig. 1A). This result extends the finding that poststress administration of quetiapine (10 mg/kg) for 21 days reverses the stress-induced decrease in hippocampal neurogenesis to its prestress levels (Luo et al., 2005). Chronic administration of 10 mg/kg quetiapine blocked the decrease in hippocampal BDNF expression caused by CRS (Fig. 1B). This is consistent with previous findings that quetiapine attenuated the stress-induced decrease in hippocampal levels of BDNF (Xu et al., 2002), and that administration of quetiapine resulted in a marked elevation of BDNF mRNA levels in the rat hippocampus under conditions of reduced NMDA receptor activity caused by MK-801 (Fumagalli et al., 2004). Chronic administration of 5 mg/kg quetiapine had no effect on the stress-induced decrease in hippocampal BDNF expression (Fig. 1B), although the same dose effectively blocked the stress-induced decrease in BrdU-labeled cells (Fig. 1A). This result suggests that quetiapine regulates hippocampal cell proliferation and BDNF expression in stressed rats through different mechanisms.

Venlafaxine dose-dependently blocked the decreases in hippocampal cell proliferation and BDNF expression caused by CRS (Fig. 2). These results extend previous findings that chronic administration of venlafaxine increased hippocampal BDNF expression (Xu et al., 2003), stimulated hippocampal cell proliferation (Khawaja et al., 2004), and reversed the decrease in hippocampal cell proliferation and BDNF levels caused by CRS (Xu et al., 2004). Our results are also in accordance with previous observations that antidepressants increased BDNF levels of patients with depression as compared

to drug-naïve patients (Chen et al., 2001). In a more recent clinical report, chronic venlafaxine treatment increased serum BDNF levels in patients with a major depressive disorder (Aydemir et al., 2005).

For the first time, we found that the combination of 5 mg/kg quetiapine and 2.5 mg/kg venlafaxine produced synergetic effects in blocking the stress-induced decrease in hippocampal cell proliferation and BDNF expression (Fig. 3A, C). This result suggests that hippocampal cell proliferation and BDNF expression are common targets for these two drugs. Although the present study did not address the underlying mechanisms for the synergistic effects, we propose that both quetiapine and venlafaxine normalize hypothalamic-pituitary-adrenal (HPA) axis activity of stressed rats. Our hypothesis is based on several findings. First, it has been demonstrated that CRS produces significant increases in HPA axis activity, which in turn, damage the hippocampus (Watanabe et al., 1992; Magarinos and McEwen, 1995; Murakami et al., 2005; Radley et al., 2005). Second, antidepressants, including venlafaxine, normalize HPA function in animals and humans (Rowe et al., 1997; Dinan, 2001; Stout et al., 2002; Nikisch et al., 2005). Third, quetiapine has been shown to reduce nocturnal urinary cortisol excretion in healthy subjects (Cohrs et al., 2004) and treat steroid-induced mania (Siddiqui et al., 2005). Also, other atypical antipsychotics normalize the HPA axis, which is related to negative symptoms of patients with schizophrenia (Aleman, 2005; Zhang et al., 2005). Finally, the combination of venlafaxine and quetiapine produced synergistic effects in preventing the CRS-induced decrease in hippocampal levels of hemeoxygenase-2 (HO-2), (Chen et al., 2005), a constitutive isoform of HO, which catalyzes the cleavage of the heme ring to form ferrous iron, carbon monoxide, and biliverdin (a potent antioxidant) (Stocker et al., 1987). Previous studies have shown that prolonged exposure to adrenal glucocorticoids decreases HO-2 levels in the hippocampus (Weber et al., 1994; Stocker et al., 1987).

The combination of 10 mg/kg quetiapine and 5 mg/kg venlafaxine produced effects comparable to those produced by either drugs alone (Fig. 3C, D). This phenomenon suggests the existence of ceiling effects and further supports that hippocampal cell proliferation and BDNF expression are the common targets of quetiapine and venlafaxine.

The finding that quetiapine and venlafaxine share hippocampal cell proliferation and BDNF expression as common targets is intriguing and is in accordance with recent advances in the understanding of hippocampal pathology. It provides an explanation for why combinations of atypical APDs and ADs are more effective than either drug type alone in treatment-resistant depression patients and in schizophrenia patients with depressive symptoms (Silver and Nassar, 1992; Hirose and Ashby, 2002). In fact, a number of animal studies have associated hippocampal BDNF expression and neurogenesis with learning, memory, and antidepressant effects (Scaccianoce et al., 2003; Bjornebekk et al., 2005; Jiang et al., 2005). Our finding may encourage further clinical studies to develop new therapeutic approaches for treatment-resistant depression patients and schizophrenia patients with depressive symptoms.

It should be noted that the changes in hippocampal cell proliferation and BDNF expression of stressed rats treated with quetiapine, venlafaxine, or their combinations are not parallel. Specifically, 5 mg/kg quetiapine effectively blocked the stress-induced suppression of hippocampal cell proliferation, but had no effect on BDNF expression in the same rats (Fig. 1); combinations of quetiapine and venlafaxine increased the number of BrdU-labeled cells as compared to the NS group, whereas the treatments kept hippocampal levels of BDNF at nonstress levels (Fig. 3). This divergence is not surprising, as BDNF is only one of many upstream molecules that enhance neurogenesis, which also includes insulin-like growth factor-1 and S100β (Aberg et al., 2003; Kleindienst et al., 2005). Also, as mentioned, the normalization of HPA axis by quetiapine and venlafaxine may be another main contributor to the regulation of hippocampal cell proliferation in the present experimental paradigm.

The present study did not include as comparisons animals who received quetiapine or venlafaxine treatment in the absence of CRS, as we do not wish to address the question of whether quetiapine and venlafaxine have additive or synergistic effects on neurogenesis in normal individuals, without depression or psychosis. In support of this notion, quetiapine has been shown to increase hippocampal BDNF mRNA levels of rats treated with MK-801, but not in normal rats (Fumagalli et al., 2004). As for venlafaxine, previous studies have shown that this drug increases hippocampal BDNF levels (Xu et al., 2003) and stimulates hippocampal cell proliferation (Khawaja et al., 2004) in normal rats.

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