Effects of quetiapine on the brain-derived neurotrophic factor expression in the hippocampus and neocortex of rats

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Abstract

The effects of antipsychotics on the brain-derived neurotrophic factor (BDNF) expression have been controversial. This study aimed to investigate the effects of chronic quetiapine administration on the BDNF mRNA expression in hippocampus and neocortex of rats with or without immobilization stress. The chronic administration (21 days) of quetiapine (10 mg/kg) significantly attenuated the decreased BDNF mRNA expression in the both hippocampal and cortical regions of rats caused by immobilization stress, and significantly increased the BDNF mRNA expression in the dentate gyrus of rats even without the immobilization stress. These results could add some theoretical bases to explain why quetiapine may improve cognitive symptoms of schizophrenia by stimulating BDNF mRNA expression.

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Brain-derived neurotrophic factor (BDNF) is the most abundant neurotrophin in the brain. BDNF regulates neuronal cell survival, differentiation, synaptic strength and morphology [6], and evidence is emerging suggesting a role in several neuropsychiatric disorders including schizophrenia [1]. Recent postmortem studies have shown that BDNF mRNA is reduced in the hippocampus and prefrontal cortex of patients with schizophrenia [20]. There has been accumulating data on the effects of antipsychotics on the BDNF expression, while the results have been conflicting [2–4,9]. The discrepancy might be due to the study design such as duration of antipsychotics administration, differences in the antipsychotics used, whether stress was given or the brains examined, and so on.

Quetiapine is an atypical antipsychotic drug showing efficacy against various symptoms of schizophrenia [16]. Chronic administration of quetiapine attenuated the decrease in rat hippocampal BDNF levels caused by immobilization stress [21]. A single dose of quetiapine also increased BDNF mRNA expression in the rat hippocampus treated with MK-801 (an antagonist of the glutamate NMDA receptor), but not in the cortical areas [5]. These two studies adopted paradigms that produced abnormal states in the brain, which were relevant as the abnormal expression of this neurotrophin has been implicated in schizophrenia [18]. However, it is unclear whether quetiapine has effect on BDNF expression in a normal state. In addition, it is also unclear that chronic administration of quetiapine also might have effect on BDNF expression in cortical regions other than hippocampus. In these contexts, the present study aimed to investigate the effects of chronic quetiapine administration on the BDNF mRNA expression in hippocampus and neocortex of rats with or without immobilization stress.

Quetiapine was generously supplied by AstraZeneca (UK). For in situ hybridization, DIG RNA labeling kit and DIG Nucleic Acid Detection kit were purchased from Roche Molecular Biochemicals (Roche, Germany). All other chemicals were purchased from Sigma (St. Louis, MO, USA). All animal manipulations were conducted in accordance with the animal care guideline of the National Institute of Health (Bethesda, MD, USA) and the Korean Academy of Medical Sciences (Seoul, Republic of Korea).

Male Sprague–Dawley rats (Hyocang Lab, Daegu, Korea) weighing 250–300 g were used. They were housed (three per cage) and maintained at 21 °C on a 12:12 h light:dark cycle with food and water freely accessible. After 7 days of acclimatization,
the rats were randomly divided into four groups of eight rats each. The first group was used as controls that received 0.8% glacial acetic acid as vehicle (1 ml/kg, i.p.) without immobilization stress (Vehicle). The second group was treated with quetiapine (10 mg/kg, i.p.) without immobilization stress (Quetiapine). The third group was treated with 0.8% glacial acetic acid as vehicle (1 ml/kg, i.p.), and 1 h later were subjected to immobilization for 2 h in specifically designed plastic tubes (Vehicle + Stress). The fourth group was subjected to the same manipulations as those in the third group except for injection with p. injection of 10 mg/kg of quetiapine (Quetiapine + Stress). These procedures were repeated once daily for 3 weeks. The dimensions of the plastic tubes in our experiment are: 20 cm/height; 7 cm/diameter.

The rats were sacrificed 24 h after the last immobilization session. Rats were deeply anesthetized with pentobarbital (75 mg/kg, i.p.) and transcardially perfused with ice-cold phosphate-buffered saline (PBS, pH 7.4) followed by ice-cold 4% paraformaldehyde in PBS. Their brains were removed, post-fixed in the same fixative for 2 h, and then cryoprotected in 15% sucrose-PBS for overnight. They were then frozen by immersion in isopentane cooled at −70 °C and stored at −80 °C until use. Serial tissue sections were cut on a cryostate (10−20 μm) and thaw-mounted on clean RNase free slides (DAKO BioTek Solution, USA).

Before acetylation, sections were fixed with 4% paraformaldehyde in PBS for 10 min. The acetylation was carried out in 0.25% (v/v) acetic anhydride in 0.1 M triethanolamine-HCl (pH 8.0) at RT for 10 min. BDNF sense and antisense RNA probes were labeled with digoxigenin-11-UTP using DIG RNA labeling kit. After ethanol dehydration, sections were hybridized in a hybridization buffer (50% deionized formamide, 300 mM NaCl, 1 × Denhardt’s solution, 50 mM Tris-Cl pH 8.0, 2 mM EDTA, 10% dextran sulphate, and 0.25 mg/ml yeast tRNA) with 200 ng/ml of either antisense or sense BDNF cDNA probe. The hybridization was performed overnight at 58 °C in a chamber humidified with 50% deionized formamide/4 × SSC. After RNase A (20 μg/ml) treatment at room temperature, non-specifically hybridized probe was washed away through several posthybridization steps in a shaking water bath starting in 2 × SSC and ending with a high-stringency washing in 0.1 × SSC at 60 °C. A final wash in 0.5 × SSC was done at room temperature. Subsequently, bound probe was detected with 1:500 alkaline phosphatase-conjugated antidigoxigenin antibody and developed into color in a BCIP/NBT solution by applying the DIG Detection Kit.

The levels of BDNF mRNA were analyzed with image analysis software (Image-Pro Plus version 3.0). The area covered with purple color was measured using an Olympus (Japan) microscope. The regions that were analyzed for in situ hybridization were three hippocampal (CA1 and CA3 pyramidal and dentate gyrus granule cell layers), and two cortical (parietal and piriform cortex), areas. These regions were analyzed by outlining the area of interest; an equivalent area was outlined for each sample. For each animal, the optical density measurements from both sides of 10 individual sections were analyzed, yielding 20 determinations, from which the mean was calculated. Average percentage values were expressed as percentage of vehicle control values. To determine the individual and interactive effects of quetiapine administration and immobilization stress on the BDNF mRNA levels, two-way ANOVA was performed. For the post hoc comparison, Dunnett’s test or t-test were followed as appropriate. Value were considered significant at p < 0.05.

Quantitative analysis of BDNF mRNA expression showed significant results in the CA1, CA3, and dentate gyrus of the hippocampal region (Fig. 3). Post hoc tests revealed that chronic immobilization stress significantly decreased BDNF mRNA expression in the three regions of hippocampus (CA1 = 30%, CA3 = 27%, and dentate gyrus = 32%; all p < 0.01). However, these conditions were reversed by the chronic administration of quetiapine that significant increases of BDNF mRNA levels were observed in the three regions (all p < 0.01). Compared to the vehicle controls, quetiapine administration without immobilization stress significantly increased the BDNF mRNA levels in the dentate gyrus (p < 0.01).

In the neocortex region, chronic immobilization stress reduced BDNF mRNA expression 71% in parietal cortex (p < 0.01) and 35% in piriform cortex (p < 0.05) compared with vehicle controls (Fig. 4). The decreased levels of the BDNF mRNA expression were also attenuated by the chronic administration of quetiapine in the both two regions (all p < 0.01). The chronic quetiapine treatment without immobilization stress did not affect the expression of BDNF.

Principal findings of the present study were that the chronic administration of quetiapine significantly attenuated the decreased BDNF mRNA expression in the both hippocampal and cortical regions of rats caused by immobilization stress, and significantly increased the BDNF mRNA expression in the dentate gyrus of rats even without the immobilization stress.

Strengths in the design of the present study were that quetiapine was administered chronically (21 days) to the rats with and without immobilization stress, and the BDNF mRNA expression was measured both in the hippocampal and cortical regions in a study. However, a weakness of the study was that the levels of the BDNF protein were not examined. Additional studies, such as RT-PCR or real-time PCR, are needed to the current effort in order to put the strong evidence on this transcript level.

The present finding that quetiapine attenuated the stress-induced decrease of BDNF expression in hippocampus was consistent with the previous reports [5,21]. It has been reported that stress is correlated with the first episode onset of schizophrenia [17] and lower levels of BDNF was found in schizophrenic patients [20]. Since quetiapine can block stress-induced decreases in BDNF mRNA, early intervention with quetiapine might prevent neurotrophin decreases induced...
by these events in vulnerable persons during first-episode schizophrenia [4].

A particular finding of the present study was that the chronic quetiapine administration blocked the stress-induced decrease of BDNF expression in the parietal and piriform cortex. This result was inconsistent with a previous study, which stated that quetiapine didn’t elevate the BDNF mRNA levels under the conditions of reduced NMDA receptor activity in cortical regions [5]. This discrepancy might be attributed to the different experiment paradigms or to the different brain areas examined in the present and previous studies. Quetiapine administered in a single dose in the previous study, while it was administered chronically for 21 days in the present study. The effects of quetiapine might be different according to the duration of administration. The examine brain regions were prefrontal and frontal cortex in the previous study, while they were parietal and piriform cortex in the present study.

The current study revealed that quetiapine increased the expression of BDNF mRNA in the dentate gyrus of rat hippocampus even under basal conditions. This finding was in agreement with a recently published study that assessed BDNF mRNA levels after a 28-day treatment with clozapine and olanzapine [3], which are atypical antipsychotics as quetiapine. Previous studies reported that typical antipsychotics such as haloperidol decreased the BDNF mRNA expressions [3,13]. The different effects between the atypical and typical antipsychotics on the BDNF expressions could be explained two ways. First, there might be links between dopamine receptor stimulation with BDNF synthesis. It has been reported that exogenous administration of the indirect dopamine agonist levodopa increases the expression of BDNF mRNA, while it was blocked by coadministration of dopamine antagonist haloperidol [12]. The atypical antipsychotics had the propensity to dissociate rapidly from D2 receptors and to allow normal dopamine neurotransmission [8]. Secondly, 5-HT system might be involved in the BDNF expressions. The pretreatment with the selective 5-HT2A receptor antagonist ketanserin significantly blocked the stress-induced down regulation of BDNF expression [19]. The atypical antipsychotics such as clozapine and quetiapine are also 5-HT2A receptor antagonists [8], while typical antipsychotics have no action on the 5-HT system.

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<td>4.41 0.037 5.40 0.021</td>
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Table 1: Summary of two-way analysis of variance.
Fig. 2. Photomicroscopic images of BDNF mRNA expression in rat neocortex. Insert shows a rat atlas [14] indicating areas where was measured. Rats were given daily injection of vehicle and quetiapine (10 mg/kg) for 21 days with or without immobilization stress (2 h daily for 3 weeks). Images represent parietal cortex (A) and piriform cortex (B). Scale bar = 200 μm.

Fig. 3. Quantitative analysis of BDNF mRNA in rat hippocampus. Rats were given daily injection of vehicle and quetiapine (10 mg/kg) for 21 days with or without immobilization stress (2 h daily for 3 weeks). Levels of BDNF mRNA in the CA1, CA3, and dentate gyrus were determined by quantitative densitometry. Three fields from each section and 10 sections from each animal were counted. The results are expressed as percentage of vehicle control levels and represent the mean ± S.E.M. of eight animals per group. *p < 0.01 vs. vehicle controls, †p < 0.01 vs. vehicle + stress animals.

Fig. 4. Quantitative analysis of BDNF mRNA in rat neocortex. Rats were given daily injection of vehicle and quetiapine (10 mg/kg) for 21 days with or without immobilization stress (2 h daily for 3 weeks). Levels of BDNF mRNA in the parietal cortex and piriform cortex were determined by quantitative densitometry. Two fields from each section and 10 sections from each animal were counted. The results are expressed as percentage of vehicle control levels and represent the mean ± S.E.M. of eight animals per group. *p < 0.05 vs. vehicle controls, **p < 0.01 vs. vehicle controls, †p < 0.01 vs. vehicle + stress animals.
BDNF is important in long-term potentiation of hippocampal neurons and in learning and memory [7,10], and its loss has been implicated in the failure of cognitive functions in dementia and Alzheimer’s disease [15]. A recent study also reported that quetiapine reverses the suppression of hippocampal neurogenesis caused by restraint stress [11]. Our findings could add some theoretical basis that atypical antipsychotics quetiapine would improve cognitive symptoms of schizophrenia by stimulating BDNF mRNA expression.

References