Differential Effects of Ziprasidone and Haloperidol on Immobilization-Stress-Induced CRF mRNA Expression in the Hypothalamic Paraventricular Nucleus of Rats

Sung Woo Park a  Sang Mi Choi b  Jung Goo Lee a–c  Chan Hong Lee a
Sun Jung Lee a  Na Ri Kim b  Young Hoon Kim a–d

a Paik Institute for Clinical Research, b Department of Psychiatry, c Haeundae Paik Hospital, School of Medicine, and d FIRST research group, Inje University, Busan, Republic of Korea

Key Words
Ziprasidone · Haloperidol · Corticotropin-releasing factor · Immobilization stress

Abstract
Objectives: Corticotropin-releasing factor (CRF) plays a prominent role in mediating the effect of stressors on the hypothalamic-pituitary-adrenal axis. In this study, we examined the effects of chronic administration of second-generation antipsychotic drug ziprasidone on CRF mRNA expression in the hypothalamic paraventricular nucleus (PVN) of rats with or without immobilization stress. Methods: The rats were subjected to immobilization stress 2 h/day for 3 weeks. The effect of ziprasidone (2.5 mg/kg, 21 days) on CRF mRNA expression was determined using in situ hybridization of tissue sections from the rat hypothalamic PVN. Haloperidol (1.0 mg/kg, 21 days) was used for comparison. Results: Haloperidol increased the expression of CRF mRNA in the PVN under basal conditions, whereas ziprasidone had no effect. Chronic immobilization stress increased CRF expression. The chronic administration of ziprasidone prevented the increase in CRF mRNA expression caused by immobilization stress. Conclusions: These results suggest that ziprasidone may have a regulatory effect on the stress-induced CRF mRNA expression and a role in the treatment of depressive mood symptom.

Introduction

Altered stress hormone regulation is frequently observed in cases of depression and anxiety [1]. In particular, many depressed patients have hypercortisolism, which is a result of hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is modulated by the hypothalamic secretion of corticotropin-releasing factor (CRF), which is one of the major physiological regulators of the stress reaction [2]. In stressful circumstances, CRF is secreted from the hypothalamus; this in turn activates the secretion of adrenocorticotropic hormone (ACTH) from the pituitary and glucocorticoids from the adrenal cortex [3]. There is strong evidence that modulation of the central CRF system provokes stress-response-associated behaviors. The central administration of CRF in rats produces behaviors associated with anxiety [4, 5], whereas CRF antagonists act as anxiolytics [6, 7]. Mice
deficient in CRF receptor 1 show decreased anxiety-like behavior and have an impaired stress response [8]. In addition, markers of CRF hyperactivity such as CRF hypersecretion and abnormal HPA axis functioning have been observed in patients with depression and schizophrenia [9]. Studies of patients with depression and animal models of depression have demonstrated that antidepressants normalize HPA axis hyperactivity and decrease the expression of the CRF gene in the paraventricular nucleus (PVN) of the hypothalamus [10–12]. Moreover, Zobel et al. [13] reported a possible antidepressant effect of CRF receptor 1 antagonists.

Second-generation antipsychotics (SGAs) like olanzapine, clozapine and ziprasidone showed efficacy in treating schizophrenic symptoms such as positive, negative and cognitive symptoms superior to that of first-generation antipsychotics (FGAs) like haloperidol [14]. In addition to beneficial treatment of schizophrenia, SGAs have recently been reported to have effects in the treatment of depression. Quetiapine has been approved for bipolar depression [15], and aripiprazole was approved as an augmentation medication in the treatment of unipolar depression by the United States Food and Drug Administration [16]. Ziprasidone has higher affinity for monoamine transporter proteins than other SGAs and ability to serotonin and norepinephrine reuptake inhibition [17]. In addition, ziprasidone has activities on 5-HT1A and 5-HT2A receptors. So, ziprasidone may be related to antidepressant mechanism [18]. According to Barbee et al. [19], 10% of the treatment-resistant depressive patients responded to ziprasidone therapy. Furthermore, ziprasidone has been shown to augment the effects of selective serotonin reuptake inhibitors in the treatment of refractory unipolar depression [20] and to have adjunctive therapeutic effects in treatment-resistant depression [21]. As mentioned earlier, CRF is a regulatory factor in HPA axis activity and in the pathophysiology of depression and anxiety. In healthy subjects, ziprasidone decreased cortisol excretion [22]. However, no study has examined the effects of ziprasidone on stress-induced CRF. Therefore, we investigated the effects of ziprasidone on stress-induced CRF mRNA expression in the rat hypothalamus. Haloperidol, an FGA, was used for comparison between FGAs and SGAs.

Materials and Methods

Animals and Drug Administration

All animal manipulations were performed in accordance with the animal care guidelines of the US National Institutes of Health (NIH publication No. 23–85, revised 1996) and the Korean Academy of Medical Science. Approval for the animal experiments was obtained from the Committee for Animal Experimentation of the Institutional Animal Laboratory Review Board of Inje Medical College (approval No. 2006–011). Chronic stress-induced down-regulation as well as antidepressant-induced upregulation of hippocampal brain-derived neurotrophic factor and neurogenesis have largely contributed to the neurotrophic hypothesis of depression. We chose this model based on past data demonstrating that repeated immobilization stress over 3 weeks but not 1 week causes reduction in the rat hippocampal neurogenesis and brain-derived neurotrophic factor mRNA expression, and these stress-induced decreases were prevented by a clinically effective antidepressant [23, 24]. Experiments were carried out on 7- to 8-week-old male Sprague-Dawley rats (KoaTech, Pyeongtaek, Korea) weighing 190–200 g on arrival at the vivarium. They were housed 3 per cage (39 × 24 × 17 cm) for the duration of the experiments. They were kept in an environment maintained at a constant temperature (21 ± 1 °C) and humidity (60 ± 10%) with a 12:12 light:dark cycle (lights on 07:00–19:00 h) and free access to food and water. The rats were allowed at least 1 week to habituate to the animal colony and weighed 250–300 g when the experimental procedures began. They were randomly assigned to the various experimental groups (n = 6/group) and weighed every week. Ziprasidone and haloperidol were dissolved in 0.4% glacial acetic acid. The first group (vehicle) was used as control and received 0.4% glacial acetic acid as vehicle (1.0 mg/kg, i.p.) without immobilization stress. The second (haloperidol) and third (ziprasidone) groups received haloperidol (1.0 mg/kg, i.p.) and ziprasidone (2.5 mg/kg, i.p.), respectively, in the same volume of vehicle without immobilization stress. Both drugs were prepared fresh daily before use and administered at 10:00 h. The fourth group (vehicle + stress) received vehicle, and then 1 h later, the rats were immobilized for 2 h (from 11:00 to 13:00 h) in specially designed plastic restraint tubes (dimensions: 20 cm long, 7 cm diameter). The rats in the fifth (haloperidol + stress) and sixth (ziprasidone + stress) groups received haloperidol (1.0 mg/kg, i.p.) and ziprasidone (2.5 mg/kg, i.p.), respectively, and were then immobilized in a similar fashion as the fourth group. These procedures were repeated once daily for 3 weeks. The dosages of these drugs used in this study were based on previous animal research [25–27] and were also comparable to the published reports based on receptor occupancy on dopamine receptors. The doses for haloperidol and ziprasidone were calculated based on animal studies by Schotte et al. [28] and Barth et al. [29], respectively. Ziprasidone was generously supplied by Pfizer Pharmaceuticals (New York, N.Y., USA) and haloperidol was purchased from Sigma (St. Louis, Mo., USA).

In situ Hybridization

The CRF mRNA was analyzed using in situ hybridization, as described previously [30]. The rats were deeply anesthetized with pentobarbital (75 mg/kg, i.p.) 24 h after the last immobilization session and perfused transcardially with ice-cold phosphate-buffered saline (PBS, pH 7.4) and ice-cold 4% paraformaldehyde in PBS. Their brains were removed, postfixed in the same fixative for 2 h and then cryoprotected in 15% sucrose-PBS overnight. Then, they were frozen by immersion in isopentane cooled to −80°C and stored at this temperature until use. Serial tissue sections were cut on a cryostat (10–20 μm). The rat CRF clones, produced by PCR using CRF-gene specific oligonucleotide primers, were identified by dideoxynucleotide sequencing. A 720-bp CRF
cRNA probe was transcribed from exon 2, which encodes the pro-CRF peptide and the mature 41-aa CRF peptide, and was labeled with digoxigenin-11-UTP using a DIG RNA labeling kit (Roche, Wyningen, Germany). After ethanol dehydration, the sections were hybridized overnight at 58 °C in hybridization buffer with 200 ng/ml of either an antisense or sense CRF cRNA probe. After RNase A treatment (20 μg/ml) at room temperature, the nonspecifically bound probe was washed away in several posthybridization steps starting in 2 × SSC and ending with a high-stringency wash in 0.1× SSC at 60 °C. A final wash in 0.5× SSC was performed at room temperature. Subsequently, the bound probe was detected with 1:500 alkaline phosphatase-conjugated anti-digoxigenin antibody, with color developed in BCIP/NBT solution using the DIG Detection Kit (Roche).

Quantification of CRF mRNA Hybridization Signals

The expression of CRF mRNA was quantified using image analysis software (Image-Pro Plus version 3.0; Media Cybernetics, Baltimore, Md., USA). Images were captured under an Olympus microscope (Tokyo, Japan) fitted with a digital camera (Nikon, Japan), and the cell density in the colored (labeled) area was counted. The PVN in the hypothalamus region was studied for in situ hybridization. This region was analyzed by outlining the area of interest; an equivalent area was outlined for each sample. Optical density measurements were made on both sides of 10 individual sections, resulting in 20 determinations, for which the mean was calculated. Average percentage values were expressed as a percentage of the vehicle control values.

Statistical Analysis

A 2-way analysis of variance (ANOVA) was performed to determine the individual and interactive effects of drug administration and immobilization stress on the CRF mRNA levels. To determine statistical differences among groups, analyses were carried out using 1-way ANOVA. For the post hoc comparison, Scheffé’s test was used as appropriate. All data are presented as means ± SEM and p < 0.05 was the accepted level of significance.

Results

Figure 1 shows representative PVN brain sections from the 6 experimental groups after hybridization with digoxigenin-11-UTP-labeled CRF RNA probes. The 2-way ANOVA showed a significant interaction of the drugs and stress (haloperidol and stress: F = 21.84, p < 0.001; ziprasidone and stress: F = 23.95, p < 0.001). The individual effects of haloperidol (F = 112.07, p < 0.001) and stress (F = 19.69, p < 0.001) were also significant, but not those of ziprasidone (F = 3.35, p = 0.07). The CRF mRNA expression as a percentage of the control was as follows: vehicle = 100, haloperidol = 170, ziprasidone = 110, vehicle + stress = 139, haloperidol + stress = 180, and ziprasidone + stress = 114 (fig. 2). The chronic administration of haloperidol significantly increased the CRF mRNA levels in the PVN of the hypothalamus of the un-stressed groups (p < 0.01), whereas difference in CRF expression between the ziprasidone and vehicle groups were not significant (p = 0.87). Chronic immobilization stress produced a significant increase in CRF mRNA expression (p < 0.01) compared to the controls. Moreover, the stress-induced elevation of CRF mRNA expression was blocked by treatment with ziprasidone (p < 0.05). Conversely, the haloperidol + stress group had significantly higher values than the stress group (p < 0.01).

Discussion

Our principal finding was that the chronic administration of ziprasidone significantly attenuated the elevated CRF mRNA expression caused by immobilization stress in the hypothalamic PVN of rats. However, ziprasidone did not affect the CRF mRNA expression in the absence of immobilization stress. Ziprasidone has a unique receptor profile that includes high-affinity antagonist activity at serotonin 5-HT3 and 5-HT4 receptors, potent agonist activity at 5-HT1A receptors and a relatively high affinity for 5-HT [31]. Some possible mecha-
neuropsychological effects based on the pharmacological properties. The decreased cortisol concentrations observed after administering olanzapine and clozapine to patients with schizophrenia were attributable to the 5-HT2A receptor antagonistic effect of olanzapine and clozapine [32–35]. Recently, Heisler et al. [36] reported that serotonin activates the HPA axis via the 5-HT2C receptor. Therefore, ziprasidone might reduce HPA axis hyperactivity.

More specifically, chronic haloperidol administration strongly increased the CRF expression under basal conditions, and this elevation was significantly higher than the stress-induced increase in CRF expression (p < 0.01). This finding is inconsistent with previous studies that assessed HPA axis activity by a single dose (3 mg) after experimentally induced heat stress [37], ACTH or cortisol levels by administration (3 mg) for 4 days in healthy human volunteers [38], and CRF mRNA expression by oral treatment (1 mg/kg) for 2 or 8 weeks in the PVN of rats [39]. This discrepancy might be attributable to the different experimental paradigms. The time point, such as 1, 2, 4 and 8 weeks, may be important in the regulation of CRF mRNA by haloperidol. Thus, these effects need to be tested at additional time points in order to confirm the reported effects. A possible mechanism by which haloperidol could upregulate CRF mRNA expression might be explained by the cAMP-protein kinase A pathway via its ability to inhibit the dopamine D2 receptor [40]. Inhibition of the dopamine D2 receptor increases the levels of cAMP, activates cAMP-protein kinase A and subsequently activates the cAMP response element-binding protein (CREB), which regulates the CRF gene [41, 42]. The CRF promoter contains a cAMP-responsive element, to which phosphorylated CREB binds and elevates transcription [43].

For haloperidol and ziprasidone, the effect of drug + stress on CRF mRNA expression was identical to that of the drug alone, which suggests identical conclusions. Although the CRF mRNA expression in both drug conditions was the same with stress as with the corresponding drug alone, it should be emphasized that stress alone increased the levels beyond those of ziprasidone, whereas haloperidol alone had already caused the CRF mRNA levels to be elevated beyond those caused by stress. However, haloperidol still might have had a stress-reducing effect, since one would have expected that the stress effect would have been added to the haloperidol effect, i.e., if haloperidol had no stress-reducing effect, the haloperidol + stress column should have been much larger than that with haloperidol alone. This cannot be tested, however, since we used separate groups without measuring the baseline scores before treatment, and the groups might simply have differed in their original levels of CRF mRNA.

The determination of the plasma levels of CRF, ACTH and corticosterone, and the examination of CRF mRNA expression in other areas of the hypothalamus is of great interest. A weakness of this study was that the effects of ziprasidone and haloperidol on additional parameters to evaluate HPA axis activity were not examined. Thus, additional studies as described above are needed for the current effort in order to put strong evidence on the CRF transcript level.

HPA axis dysfunction is observed in patients with psychiatric illness, including depression and schizophrenia [44, 45]. The administration of ziprasidone and quetiapine
pine decreases the urinary cortisol excretion in healthy subjects [22, 46]. Olanzapine also reduces the ACTH and cortisol secretion in healthy subjects, whereas haloperidol has no such affect. Olanzapine and clozapine reduce the cortisol levels, which are associated with improved psychopathology in patients with schizophrenia [35, 47]. Moreover, a recent study reported that the potential benefit from using SGAs in mood disorders, including depression, seems to be clinically justified [48]. These data suggest that some SGAs, including ziprasidone, might improve depressive symptoms by normalizing HPA axis activity. This study is the first report on the differential effects of ziprasidone and haloperidol on CRF mRNA expression in rats with or without immobilization stress. The significant differences in the actions of ziprasidone and haloperidol may explain the possible antidepressant-like effect of ziprasidone.

**Acknowledgment**

This work was supported by a Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2005-041-E00241).

**Ziprasidone and CRF mRNA Expression**

**Neuropsychobiology** 2011;63:29–34

**References**


