Effects of some psychiatric drugs on **corticotropin-releasing factor (CRF)** gene expression in mouse hypothalamus cells *in vitro*

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**ABSTRACT**

Psychiatric drugs are typically used to treat patients suffering from mental disorders and some conditions of stress-related disorders. Effects of psychiatric drugs actions were proposed to be involved in regulation of neuronal gene expression. One of the genes involved is **corticotropin-releasing factor (CRF)** gene that plays a critical role in controlling the hypothalamus-pituitary-adrenal (HPA) axis under stress condition. This study aims to examine the effects of psychiatric drugs on CRF gene expression in a mouse hypothalamus cell line. Quantitative real-time PCR was used to determine expression levels of CRF gene in cells treated with diazepam, risperidone and haloperidol. Results showed that all of the drugs tested tend to suppress CRF gene expression at the RNA levels, both before and after the cells were incubated with forskolin, a stress-inducing agent. The findings suggested that the mechanism of psychiatric drugs actions may act in HPA negative-feedback control by regulating CRF mRNA expression in response to stress.

**Keywords**: Psychiatric drugs; HPA; CRF

**INTRODUCTION**

Psychiatric drugs are typically used to treat patients suffering from mental disorders such as depression, anxiety, schizophrenia, bipolar disorder, obsessive compulsive disorder (OCD), and attention deficit-hyperactivity disorder (ADHD). Mental disorders may directly relate to malfunctions of the hypothalamic-pituitary-adrenal (HPA) axis stress response system (Mitchell, 1998; McGowan *et al.*, 2009; Perroud *et al.*, 2011). **Corticotropin-releasing factor (CRF)** gene is one of the important genes that play a central role in the HPA axis negative-feedback regulation (Owens and Nemeroff, 1991; Pelleymounter *et al.*, 2000; Stout *et al.*, 2002), which is potentially responsible for psychiatric disorders by mediating both the neuroendocrine and central nervous system (CNS) response to stress (Vermenet and Bremner, 2002a). Many drugs are needed to develop the efficiency of therapeutic action via some mechanisms other than neurotransmitters circulation of the brain and nervous system. Some studies have indicated the interaction between neurotransmitter systems and CRF within the direct synaptic neurons, especially dopamine-CRF interaction (Swerdlow *et al.*, 1986; Toufexis *et al.*, 2004; Meloni *et al.*, 2006). Furthermore, a few studies have demonstrated a possible alternate action of psychiatric drug effects within neurons (Vargas *et al.*, 2001; Dirks *et al.*, 2003; Park *et al.*, 2011). Therefore, it is possible that CRF gene expression regulation is one of the mechanisms related to psychiatric drugs mode of action.

Many types of psychiatric drugs are used to treat psychiatric disorders. diazepam, risperidone and haloperidol are among popular therapeutic drugs. Diazepam (or first marketed as Valium) is commonly used to reduce tension and anxiety. The pharmacological action of diazepam enhances the neurotransmitter gamma-aminobutyric acid (GABA) by stimulating the GABA<sub>A</sub> receptor (Riss *et al.*, 2008). Previous studies have shown that diazepam decreases HPA axis activity in stressful contexts (Lakic *et al.*, 1986; Pivac and Pericic, 1993), although its influences seem to increase basal HPA axis activity in rats under some certain experimental conditions (Vargas *et al.*, 2001). Moreover, Swerdlow *et al.* (1986) has found the dopamine-CRF interaction by which the anxiolytic drug chlordiazepoxide, which was regulated by the GABAergic systems, could inhibit the CRF-enhanced startle in neurons (Swerdlow *et al.*, 1986). Risperidone (trade name and generics are Risperdal) is an atypical antipsychotic drug that is mainly used to treat schizophrenia.
(Leucht et al., 2013), bipolar disorder (Muralidharan et al., 2013), and irritability in autistic patients (Sharma and Shaw, 2012; Kirino, 2014). The pharmacological action of risperidone is a dopamine antagonist by tight binding with D2/5-HT6 receptor antagonist to decrease dopamine neurotransmitter (Brunton et al., 2010). On the other hand, haloperidol (marketed under the trade name is Haldol) is a typical antipsychotic medication. This drug is also used to treat schizophrenia, acute psychosis, mania, delirium, and severe anxiety (Brayfield, 2013). The pharmacological action of haloperidol is a dopamine D2 receptor antagonist (Brayfield, 2013). A research in a transgenic mouse model of life-long CRF overproduction (CRF-OE) found that risperidone and haloperidol are effective in reversing the startle reactivity and prepulse inhibition (PPI) in the CRF-OE mouse (Dirks et al., 2003). The result of CRF system abnormalities may implicate in patients with certain aspects of several neuropsychiatric disorders where PPI is deficient, especially schizophrenia, OCD, and possibly post traumatic stress disorder (PTSD) (Braff et al., 1992; 2001; Bremner et al., 1997; Grillon et al., 1998). Interestingly, the interaction between dopamine-CRF in the bed nucleus of the stria terminalis (BSTld), which was the specific brain areas that directly receive dopaminergic inputs, has been reported (Meloni et al., 2006). The result suggested that the selective dopamine receptor antagonists on CRF-enhanced startle might have anxiety-like effects. However, there has been no report on the drug action on the downstream regulation of the hypothalamic CRF expression in stressful period that is of great interest. Previously, there are some researches tried to find about the role of some antidepressant and psychiatric drugs on the activity of HPA axis regulation through directly CRF gene promoter activity (Budziszewska et al., 2002) and CRF mRNA expression using in situ hybridization of the rat hypothalamic paraventricular nucleus (PVN) (Park et al., 2011). Therefore, the exact mechanisms of downstream target genes of psychiatric drugs action are needed to understand for further developing the therapeutic efficiency in clinical treatment for many psychiatric disorders.

This study aims to analyze actions of psychiatric drugs on the hypothalamic CRF expression in stress response, both to prevent and reduce the stress in vitro.

MATERIALS AND METHODS
Psychiatric drugs
Diazepam, risperidone and haloperidol, three representatives of psychiatric drugs, were used in this experiment. Concentrations of each psychiatric drugs used in this study are based on the reference range of the blood or plasma drug concentrations in persons receiving the drug therapeutically that have been reported: 0.5 mcg/mL for diazepam, 50 ng/mL for risperidone, and 20 ng/mL for haloperidol (Reidenberg et al., 1978; Moulin et al., 1982; Ereshefsky et al., 1984). Ten µM forskolin was used as a stress reagent, and 100 nM dexamethasone was used as a positive control to suppress CRF expression (Kageyama et al., 2008). All reagents were purchased from Sigma-Aldrich Pte Ltd, Singapore.

Hypothalamus cell culture
Immortalized mouse embryonic hypothalamic primary culture (mHypoE-N6) was purchased from CELLutions Biosystems (Cedarlane Laboratories, Canada). The cells were grown for 3-5 days or 80% confluence. Cells were plated into 6-well tissue culture plate at 10⁶ cells/well and incubated at 37 °C with 5% CO₂ overnight before each experiment.

Drug treatments
Two experiments were performed to analyze the effects of psychiatric drugs on endogenous CRF gene expression, reducing stress and preventing the cells from stress. To analyze the effects of the drugs on stress reduction, mHypoE-N6 cells were grown in 6-well/plate (60-70% confluence), incubated with DMEM containing 10 µM forskolin for 2 h (Kageyama et al., 2008), and incubated with DMEM containing either 100 nM dexamethasone (Kageyama et al., 2008), 0.5 mcg/mL diazepam, 50 ng/mL risperidone, or 20 ng/mL haloperidol for 30 min. To analyze the effects of the drugs on stress protection, mHypoE-N6 cells were pre-incubated in DMEM supplemented with 100 nM dexamethasone (Kageyama et al., 2008) and each drug (0.5 mcg/mL diazepam, 50 ng/mL risperidone, and 20 ng/mL haloperidol) for 30 min, then subsequently incubated with DMEM containing 10 µM forskolin for 2 h (Kageyama et al., 2008). All experiments were carried out in triplicate.

Quantitative RT-PCR analysis
Total RNA was extracted using the RNeasy plus Mini Kit (Qiagen, USA) and was reverse transcribed using the Senscript Reverse Transcription Kit (Qiagen). Real-time PCR was carried out in triplicate using SsoFast™ EvaGreen® supermix (Bio-Rad, USA)
following the manufacturer’s instructions. For CRF gene expression, mouse CRF primers are as followed: mCRFF, 5’-CAGGAAACTGATGGAGA TTATCG-3’ and mCRFR, 5’-AGAAATTAGCAT GGGAATA CA-3’. Mouse GAPDH was used as the normalized internal control and was amplified using primer sequences: mGAPDHF, 5’-AACCTTGGCATTGTGGAAGG-3’ and mGAPDHR, 5’-ACACATTGGGGTGGGAA CA-3’. The relative gene expressions of CRF were calculated using the 2−ΔΔCt method (Pfafl, 2001).

Statistical analysis
Relative gene expression values between groups of treated cells with forskolin-induced CRF expression were compared with each antagonistic psychiatric drugs treatment using unpaired t-test (Microsoft® Excel).

RESULTS AND DISCUSSION
Effects of each selected psychiatric drugs on CRF gene expression in hypothalamic cell lines are shown in Figures 1 and 2. Cells treated with medium alone and medium with forskolin were used as controls. Dexamethasone was used as a positive control. Results showed that all of psychiatric drugs tend to suppress CRF gene expression at the RNA level under stress both before and after the cells were incubated with forskolin, the stress reagent (Figures 1 and 2, respectively). When cells were treated with psychiatric drugs prior to forskolin, only risperidone significantly reduced CRF mRNA levels (p < 0.05) (Figure 1). Although diazepam and haloperidol tend to also protect the cells from stress, their efficacy was not significant. On the other hand, all of drugs significantly decreased the CRF expression in the cells compared with cells treated with forskolin alone, when cells were treated with drugs after forskolin (p < 0.05) (Figure 2).

Our preliminary results supported the previous findings about the effect of ziprasidone administration on the immobilization-stress-induced CRF mRNA expression (Park et al., 2011). All of three psychiatric drugs used in this study - diazepam, risperidone and haloperidol – tend to prevent cells from stress before cells were incubated with forskolin stress reagent (Figure 1), the differences were statistically significant in only risperidone efficacy. Interestingly, when cells were treated with forskolin prior to the drugs, all of drugs tested significantly suppressed CRF mRNA expression (Figure 2). Therefore, it is possible that these psychiatric drugs actions may involve the HPA axis negative feedback mechanism through CRF gene expression, as reported in earlier studies performed in Neuro-2A neuroblastoma and AtT-20 pituitary cell lines (Budziszewska et al., 2002; 2004). Previous data suggested that the cyclic adenosine monophosphate (cAMP) response element (CRE) region may play an important role in the basal upstream regulatory pathway of CRF gene (Seasholtz et al., 1988; Guardiola-Diaz et al., 1994; Itoi et al., 1996). In addition, some reports also suggested that CRF gene transcription was induced by forskolin via cAMP-stimulated CRE region on CRF promoter. In contrast, forskolin-induced CRF transcription was repressed by dexamethasone via some regions on CRF promoter (Kageyama et al., 2008). However, the exact mechanism of psychiatric drugs involved in hypothalamic CRF activity should be further identified.

![Figure 1](image-url) Effects of dexamethasone (DEXA) and three selected psychiatric drugs, diazepam, risperidone, and haloperidol, on CRF gene expression in hypothalamus cell lines before incubation with forskolin (FSK). The CRF expression levels were compared with GAPDH. *p < 0.05.
Figure 2 Effects of dexamethasone (DEXA) and three selected psychiatric drugs, diazepam, risperidone, and haloperidol, on CRF gene expression in hypothalamus cell lines after incubation with forskolin (FSK). The CRF expression levels were compared with GAPDH. *p < 0.05.

In summary, we have analyzed the effects of selected psychiatric drugs on forskolin-induced CRF gene expression in a mouse hypothalamus cell line. We found that risperidone significantly prevent cells from stress and all of the drugs tested significantly reduce the stress in the cells. Understanding of molecular mechanism of HPA axis negative feedback pathway response to stressful periods is needed for development of improved clinical treatment efficacy in many psychiatric stress-related disorders.

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