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

Matchima Nantharat[1,2], Rachaneekorn Tammachote[1,3*], Verayuth Praphanphoj[2]

[1]*Biological Sciences Program, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand.*
[2]*Center for Medical Genetics Research, Rajanukul Institute, Bangkok 10400, Thailand.*
[3]*Human Genetics Research Group, Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand.*

**Corresponding author: ratchaneekorn.t@chula.ac.th*

ABSTRACT

Psychiatric drugs are typically used to treat patients suffered 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 suffered 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 (Vermetten 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 $GABA_A$ 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 D_2/5-HT_2/α_1/α_2 receptors 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 D_2 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 10^6 cells/well and incubated at 37 ºC with 5% CO_2 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 Sensiscript 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'-AGAAATTAAGCAT GGGCAATA CA-3'. Mouse *GAPDH* was used as the normalized internal control and was amplified using primer sequences: mGAPDHF, 5'-AACTTTGGCATTGTGGAAGG-3' and mGAPDHR, 5'-ACACATTGGGGGTAGGAA CA-3'. The relative gene expressions of *CRF* were calculated using the $2^{-\Delta\Delta CT}$ method (Pfaffl, 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 hypothalamus 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.

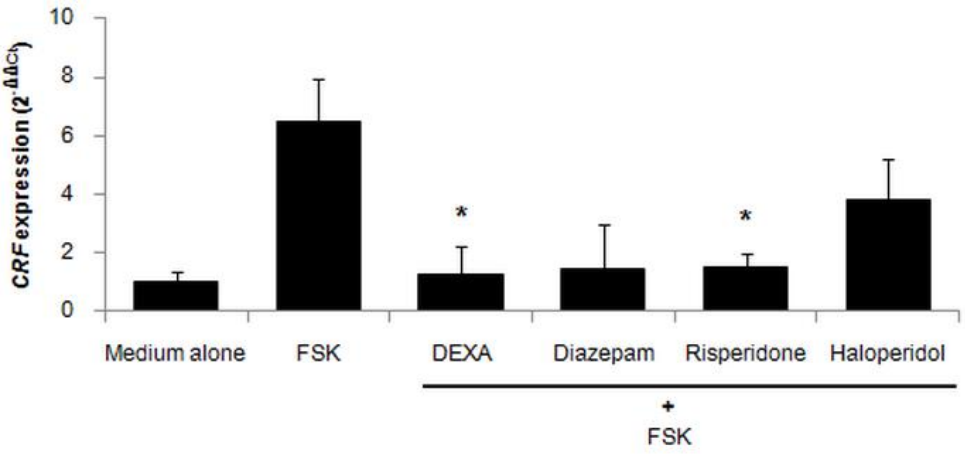

Figure 1 Effects of dexamethasone (DEXA) and three selected psychiatric drugs, diazepam, risperidone, and haloperidol, on *CRF* gene expression in hypothalamus cell lines before incubation with forskolin (FSK). The *CRF* expression levels were compared with *GAPDH*. *$p < 0.05$.

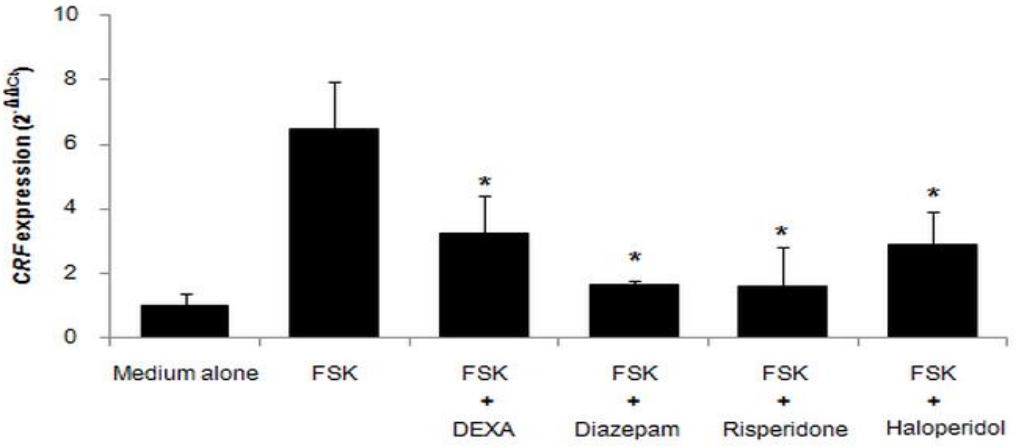

Figure 2 Effects of dexamethasone (DEXA) and three selected psychiatric drugs, diazepam, risperidone, and haloperidol, on *CRF* gene expression in hypothalamus cell lines after incubation with forskolin (FSK). The *CRF* expression levels were compared with *GAPDH*. *$p < 0.05$.

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.

ACKNOWLEDGEMENTS

This research was supported by grants from Center for Medical Genetics Research (CMGR), Thailand Center of Excellence for Life Sciences (TCELS), The 90th Anniversary of Chulalongkorn University Fund (Ratchadaphisek somphot Endowment Fund), and The Human Resource Development in Science Project (Science Achievement Scholarship of Thailand; SAST).

REFERENCES

Braff DL, Grillon C, Geyer MA (1992) Gating and habituation of the startle reflex in schizophrenic patients. Arch Gen Psychiatry 49: 206–215.

Braff DL, Geyer MA, Swerdlow NR (2001). Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. Psychopharmacology (Berl) 156: 234–258.

Brayfield A (2013) Haloperidol. Martindale: The Complete Drug Reference. Pharmaceutical Press, London, UK.

Bremner JD, Licinio J, Darnell A, Krystal JH, Owens MJ, Southwick SM, Nemeroff CB, Charney DS (1997) Elevated CSF corticotropin-releasing factor concentrations in posttraumatic stress disorder. Am J Psychiatry 154: 624–629.

Brunton L, Chabner B, Knollman B (2010) Goodman and Gilman's the pharmacological basis of therapeutics. 12th ed. McGraw Hill Professional, New York.

Budziszewska B, Jaworska-Feil L, Tetich M, Basta-Kaim A, Kubera M, Leskiewicz M, Lasoñ W (2002) Effect of antidepressant drugs on the human corticotrophin-releasing-hormone gene promoter activity in neuro-2A cells. Pol J Pharmacol 54: 711–716.

Budziszewska B, Jaworska-Feil L, Tetich M, Basta-Kaim A, Kubera M, Leoekiewicz M, Lason W (2004) Regulation of the human corticotropin-releasing-hormone gene promoter activity by antidepressant drugs in Neuro-2A and AtT-20 Cells. Neuropsychopharmacology 29: 785–794.

Dirks A, Groenink L, Westphal KGC, Olivier JDA, Verdouw PM, van der Gugten J, Geyer MA, Olivier B (2003) Reversal of startle gating deficits in transgenic mice overexpressing corticotropin-releasing factor by antipsychotic drugs. Neuropsychopharmacology 28: 1790–1798.

Ereshefsky L, Davis CM, Harrington CA, Jann MW, Browning JL, Saklad SR, Burch NR (1984) Haloperidol and reduced haloperidol plasma levels in selected schizophrenic patients. J Clin Psychopharmacol 4: 138–142.

Grillon C, Morgan CA, Davis M, Southwick SM (1998) Effects of experimental context and explicit threat cues on acoustic startle in Vietnam veterans with posttraumatic stress disorder. Biol Psychiatry 44: 1027–1036.

Guardiola-Diaz HM, Boswell C, Seasholtz AF (1994) The cAMP-responsive element in the corticotropinreleasing hormone gene mediates transcriptional regulation by depolarization. J Biol Chem 269: 14784–14791.

Itoi K, Horiba N, Tozawa F, Sakai Y, Sakai K, Abe K, Demura H, Suda T (1996) Major role of 3'5'-cyclic adenosine monophosphate-dependent protein kinase A pathway in corticotropin-releasing factor gene expression in the rat hypothalamus in vivo. Endocrinology 137: 2389–2396.

Kageyama K, Hanada K, Takayasu S, Iwasaki Y, Sakihara S, Nigawara T, Suda T (2008) Involvement of regulatory elements on corticotropin-releasing factor gene promoter in hypothalamic 4B cells. J Endocrinol Invest 31: 1079–1085.

Kirino E (2014) Efficacy and tolerability of pharmacotherapy options for the treatment of irritability in autistic children. Clin Med Insights Pediatr 8: 17–30.

Lakic N, Pericic D, Maney H (1986) Mechanism by which picrotoxin and a high dose of diazepam elevate plasma corticosterone level. Neuroendocrinology 43: 331–335.

Leucht S, Cipriani A, Spineli L, Mavridis D, Orey D, Richter F, Samara M, Barbui C, Engel RR, Geddes JR, *et al*. (2013) Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: a multiple-treatments meta-analysis. Lancet 382(9896): 951–962.

McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labonte B, Szyf M, Turecki G, Meaney MJ (2009) Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nat Neurosci 12: 342–348.

Meloni EG, Gerety LP, Knoll AT, Cohen BM, Carlezon Jr WA (2006) Behavioral and anatomical interactions between dopamine and corticotropin-releasing factor in the rat. J Neurosci 26(14): 3855-3863.

Mitchell AJ (1998) The role of corticotropin releasing factor in depressive illness: a critical review. Neurosci Biobehav Rev 22(5): 635–651.

Moulin MA, Davy JP, Debruyne D, Andersson JC, Bigot MC, Camsonne R, Poilpre E (1982) Serum level monitoring and therapeutic effect of haloperidol in schizophrenic patients. Psychopharmacol 76: 346–350.

Muralidharan K, Ali M, Silveira LE, Bond DJ, Fountoulakis KN, Lam RW, Yatham LN (2013) Efficacy of second generation antipsychotics in treating acute mixed episodes in bipolar disorder: a meta-analysis of placebo-controlled trials. J Affect Disord 150(2): 408–414.

Owens M, Nemeroff C (1991) Physiology and pharmacology of corticotropinreleasing factor. Pharmacol Rev 43: 425–473.

Park SW, Choi SM, Lee JG, Lee CH, Lee SJ, Kim NR, Kim YH (2011) Differential effects of ziprasidone and haloperidol on immobilization-stress-induced *CRF* mRNA expression in the hypothalamic paraventricular nucleus of rats. Neuropsychobiology 63: 29–34.

Pelleymounter M, Joppa M, Carmouche M, Cullen MJ, Brown B, Murphy B, Grigoriadis DE, Ling N, Foster AC (2000) Role of corticotropin-releasing factor (CRF) receptors in the anorexic syndrome induced by CRF. J Pharm Exp Ther 293: 799–806.

Perroud N, Paoloni-Giacobino A, Prada P, Olie E, Salzmann A, Nicastro R, Guillaume S, Mouthon D, Stouder C, Dieben K, *et al*. (2011) Increased methylation of glucocorticoid receptor gene (*NR3C1*) in adults with a history of childhood maltreatment: a link with the severity and type of trauma. Transl Psychiatry 1: e59.

Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29(9): e45.

Pivac N, Pericic D (1993) Inhibitory effect of diazepam on the activity of the hypothalamic-pituitary-adrenal axis in female rats. J Neural Transm 92: 173–186.

Reidenberg MM, Levy M, Warner H, Coutinho CB, Schwartz MA, Yu G, Cheripko J (1978) Relationship between diazepam dose, plasma level, age, and central nervous system depression. Clin Pharmacol Ther 23: 371–374.

Riss J, Cloyd J, Gates J, Collins S (2008) Benzodiazepines in epilepsy: pharmacology and pharmacokinetics. Acta Neurol Scand 118(2): 69–86.

Seasholtz AF, Thompson RC, Douglass JO (1988) Identification of a cyclic adenosine monophosphate-responsive element in the rat corticotropin-releasing hormone gene. Mol Endocrinol 2: 1311–1319.

Sharma A, Shaw SR (2012) Efficacy of risperidone in managing maladaptive behaviors for children with autistic spectrum disorder: a meta-analysis. J Pediatr Health Care 26(4): 291–299.

Stout SC, Owens MJ, Nemeroff CB (2002) Regulation of corticotropin-releasing factor neuronal systems and hypothalamic-pituitary-adrenal axis activity by stress and chronic antidepressant treatment. J Pharmacol Exp Ther 300: 1085–1092.

Swerdlow NR, Geyer MA, Vale WW, Koob GF

(1986) Corticotropin-releasing factor potentiates acoustic startle in rats: blockade by chlordiazepoxide. Psychopharmacology (Berl) 88: 147-152.

Toufexis DJ, Davis C, Hammond A, Davis M (2004) Progesterone attenuates corticotropin-releasing factor-enhanced but not fear-potentiated startle via the activity of its neuroactive metabolite, allopregnanolone. J Neurosci 24: 10280-10287.

Vargas ML, Abella C, Hernandez J (2001) Diazepam increases the hypothalamic-pituitary-adrenocortical (HPA) axis activity by a cyclic AMP-dependent mechanism. Br J Pharmacol 133: 1355–1361.

Vermetten E, Bremner JD (2002a) Circuits and systems in stress. II. Applications to neurobiology and treatment in posttraumatic stress disorder. Depress Anxiety 16: 14-38.