Pharmacologia 3 (11): 574-582, 2012
ISSN 2044-4648 / DOI: 10.5567/pharmacologia.2012.574.582

Combined Analysis of mRNA Expression of Dopamine Receptors D_1, D_2 and cfos in Different Brain Regions of Stressed Rats

[1,2]N. Rasheed, [2,3]A. Ahmad and [4]A. Alghasham
[1]Department of Medical Biochemistry, College of Medicine, Qassim University, Buraidah, Saudi Arabia
[2]Neuropharmacology Unit, Division of Pharmacology, Central Drug Research Institute, Lucknow, India
[3]Amity Institute of Biotechnology, Amity University, Lucknow, India
[4]Department of Pharmacology and Therapeutics, College of Medicine, Qassim University, Buraidah, Saudi Arabia

Abstract: Dopamine (DA) exerts powerful effects on the central regulation of motor and behavioral activities during stressful conditions. This study aimed to evaluate the response of dopaminergic system in Acute Stress (AS) and Chronic Unpredictable Stress (CUS) by measuring dopamine receptor (D_1 and D_2) gene expression and distribution in the frontal cortex, striatum, hippocampus and amygdala brain regions of rats and also investigated the corresponding changes in the expression of immediate early gene (cfos) in the all selected brain regions. Involvement of D_1 receptor in AS and CUS was examined by using A68930 (a D_1 selective agonist). Rats were exposed to AS (single immobilization for 150 min) and CUS (two different stressors for 7 days). The results showed that under baseline condition cfos gene was distributed in the brain, with the highest density in the frontal cortex followed by amygdala, striatum and hippocampus. We also found that AS and CUS caused an induction of cfos gene in frontal cortex, striatum and amygdala. Importantly the increased cfos mRNA expression remains up regulated following the treatment of A68930 at 0.25 mg kg^{-1} dose suggested that neurons in these brain structures are activated. D_1 receptor gene was also found to be distributed in the brain, with the highest density in the striatum followed by amygdala, frontal cortex and hippocampus. In case of D_2 receptor gene, the highest density was found in the striatum followed by amygdala, hippocampus and frontal cortex. Whereas, D_1 receptor gene was found to be elevated in the striatum and hippocampus in both stressed models, but this was normalized by A68930 treatment. On the other hand, D_1 receptor mRNA level was reduced in the frontal cortex and amygdala regions in CUS group only. Whereas, the expression D_2 receptor remains unchanged in these regions. These changes in gene expression of D_1/D_2 receptor and cfos, clearly indicating their diverse role in various central nervous system functions operated in particular brain regions.

Key words: Acute stress, chronic unpredictable stress, dopamine, dopamine receptor, cfos

INTRODUCTION

Increasing number of evidences indicate that stressful life events contribute to the expression and exacerbation of different physiological, behavioral and neurological pathologies (Nieoullon and Coquerel, 2003). However, most of these studies suggest a major involvement of stress on central dopaminergic systems, in particular mesolimbic and mesocortical systems (Rasheed *et al*., 2011; Pani *et al*., 2000). Dopamine (DA) neurons in the Central Nervous System (CNS) are thought to play a critical role in various neurological and psychiatric disorders, including schizophrenia, Parkinson's disease and drug addiction (Van Craenenbroeck *et al*., 2005). Studies on DA receptors have been a primary approach towards understanding of dopaminergic function, in turn, it may lead to the development of improved therapeutic approaches to treat these devastating neurological disorders (Hiroi *et al*., 2002). When characterized by strictly pharmacological approach, there are two major classes of DA receptors subtypes D_1 class and D_2 class subtypes (Kebabian and Calne, 1979) and it plays the key role in the control of locomotion, learning, working memory, cognition and emotion (Nieoullon and Coquerel, 2003; Benturquia *et al*., 2008). Therefore it was considered to be a major target for drug designing applied in the treatment of neuropsychiatric diseases. Stress has been shown to alter normal dopaminergic neurotransmission (Pani *et al*., 2000) and exposure to stress profoundly increases the

Corresponding Author: N. Rasheed, Department of Medical Biochemistry, College of Medicine, Qassim University, P.O. BOX 6655, Buraidah-51452, KSA Tel: +966-597274317

dopaminergic activity (Varga *et al*., 2011) and induces relevant adaptive responses of DA receptors in specific brain regions (Brunelin *et al*., 2008). In the initial part of our study, we observed a differential and brain region specific dopaminergic response in relation to the DA levels, D_1/D_2 receptor densities and consequent locomotor behavior during stressful conditions (Rasheed *et al*., 2010). However, the effect of stress and DA receptor activation by specific agonists on the expression of these vital DA receptor genes in important brain region remains to be elucidated. Thus, it was relevant to explore the stress induced-changes of D_1 /D_2 receptors at the mRNA level. In the present study, we aimed to evaluate the response of dopaminergic system in Acute Stress (AS) and Chronic Unpredictable Stress (CUS) in terms of mRNA distribution and expression of D_1 /D_2 receptors in the frontal cortex, striatum, hippocampus and amygdale of rat brain. The effect of A 68930, a D_1 selective agonist on the expression of DA receptor genes was also examined to understand the biological basis of behavioral, hormonal and other stress-induced changes observed during AS and CUS conditions. Further, This study examined the involvement of brain regions mediating the changes of DA receptor gene expression during different stressful conditions and following by the treatment of A 68930 by measuring the distribution and expression of c-fos in these brain regions to locate the precise neuronal activation.

MATERIALS AND METHODS

Animals: Experimental protocols were approved by the Institutional Ethical Committee of CDRI with following guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) which complies with International norms of INSA (Indian National Science Academy). Adult male Sprague Dawley rats, (180-220 g) procured were used in the study. For both control and stressed groups three rats were housed per cage, in a room with temperature regulated at 22±2°C, with a 12 h/12 h light/dark cycle (lights on 07:00 h, lights off 19:00 h). Standard chow pellets and water were given *ad libitum*, except during the period when food or water deprivation was applied.

Drugs: A 68930[(1R,3S)-1-aminomethyl-5,6-dihydroxy-3-phenylisochroman HCl]-a D_1 receptor agonist, was obtained from Sigma Chemicals, St Louis, MO, USA. All other chemicals used in the study were of analytical or HPLC grade purchased locally.

Treatment schedule: Animals were randomly divided into various groups, each group containing 10 rats. The, respective groups consist of Non-stress (NS), Acute Stress (AS), Chronic Unpredictable Stress (CUS) along with A 68930 treatment groups (A 68930+AS and A 68930+CUS). A 68930 was prepared in 0.9% NaCl solution. Normal control (NS) and stress control groups (AS and CUS) were injected with the same vehicle solutions as used for the dissolution of respective drugs and used for the purpose of comparison accordingly. Drugs and vehicles were administered intraperitoneally 20 min prior to stress regimen in AS and CUS conditions in a volume of 1 mL kg^{-1} b.wt.

Stress procedures: All the rats were acclimatized under laboratory conditions and handled daily for a week prior to the commencement of experiment. Two different stress models were used, Acute Stress (AS) and Chronic Unpredictable Stress (CUS). AS was produced by restraining 12 h fasted rats for 150 min inside a cylindrical steel tube (7 cm diameter, 17.5 cm long, with holes for ventilation) as previously described (Sheikh *et al*., 2007; Ahmad *et al*., 2010). CUS procedure includes fasting, tail pinching, restraint, overnight wet cage bedding, isolation, forced swimming, day-night reversal, cold-restraint, water deprivation, foot-shock and cold exposure (Katz *et al*., 1981). Individual stressors and time of exposure during CUS on every day have been summarized in Table 1. Briefly, rats were subjected to fasting (food deprivation) for 18 h, between 14.00 h to 10.00 h the next day. Tail pinching comprised pinching the tail tip with specially designed steel clips for 5 min. Restraint stress comprised confinement for 150 min inside a cylindrical steel tube (7 cm diameter, 17.5 cm long). Bedding material was soaked with water overnight as a further stressor. For isolation stress, rats were kept alone in a cage for 12 h. For swimming stress, rats were placed in a glass jar (35.5 cm high, 20.2 cm diameter) containing water (depth: 25 cm) at

Table 1: Schedule of stressors used during chronic unpredictable stress for a period of 7 days

Day	Time	Procedure	Duration	Time	Procedure	Duration (h)
1	10:00 a.m.	Tail pinching	5 min	2:00 p.m.	Fasting (food deprivation)	18
2	10:00 a.m.	Restraint stress	150 min	3:00 p.m.	Wetted soil cage	18
3	9:00 a.m.	Isolation stress	12 h	9:00 p.m.	Day night reversal	15
4	12:30 p.m.	Cold restraint stress	2 h	3:00 p.m.	Water deprivation	18
5	10:00 a.m.	Foot shock	20 min	4:00 p.m.	Cold exposure	3
6	10:00 a.m.	Forced swimming	30 min	2:00 p.m.	Fasting (food deprivation)	18
7	9:00 a.m.	Restraint stress	150 min	12:30 p.m.	Sacrificed by cervical dislocation	---

25°C for 30 min. Day and night reversal involved keeping the rats in the dark during the usual day (3 h) and in high intensity light during the night (12 h). During water deprivation, water was removed for 18 h, between 14.00 h to 10.00 h the next day. In foot-shock stress, rats were subjected for 20 min to one shock per 2 sec of 2 mA via a grid floor in an agressometer (Techno electronics, Lucknow, India). Cold exposure involved placing rats in a programmable environment test chamber (Remi, Mumbai, India) for 3 h at 4°C. In cold-restraint stress, rats were restrained as above and kept in the cold chamber for 2 h at 4°C.

Sample preparation for RT-PCR: Immediately after the last stressor all the rats were killed by conscious cervical dislocation followed by decapitation and the brains were immediately removed. The discrete regions (cortex, striatum, hippocampus and amygdala) were dissected on an ice-cold glass plate (Glowinski and Iversen, 1966). Total RNA was extracted from brain samples using TRIZOL Reagent (Invitrogen Life Technologies, Karlsruhe, Germany). First-strand cDNA was synthesized using 5 μg of total RNA using commercially available RETRO script kit (Ambion Inc, Austin, TX,USA). Genes for D_1, D_2 receptor, cfos and b-Actin were amplified with specific primer sets as previously described (Vrana *et al*., 1995; Konturek *et al*., 2003; Nakahara *et al*., 2002). cDNA samples were annealed at 94°C (5 min) and amplified for 30 cycles with the following cycling conditions: 94°C for 1 min; respective annealing temperature for D_1 receptor-60°C, D_2 receptor -63°C, c-fos -63°C and b-Actin -55°C for 1 min; 72°C for 1 min followed by a final extension at 72°C for 10 min. The amplified products were electrophoresed on 1.0% agarose gel in TAE buffer and visualized by ethidium bromide staining.

Densitometric analysis: Measurement of the scanned bands was performed using UN-SCAN-IT software (Silk Scientific Corporation, Oren, UT, USA). Each band was scanned five times and the mean band intensity (pixel per band) was obtained. Data were normalized to suitable loading controls and expressed as Mean+SD followed by appropriate statistical analysis.

Statistical analysis: All statistical analyses were performed using one-way Analysis of Variance (ANOVA) with Tukey-Kramer post-hoc analysis and $p<0.05$ was considered statistically significant. For cell counting and fibers density, inter group comparisons were performed with ANOVA values shown as Mean+SEM unless stated otherwise.

RESULTS

Effect of A 68930 on AS and CUS induced changes in brain DA D_1/D_2 receptors gene expressions

Distribution of DA D_1/D_2 receptor gene expression in selected brain regions: The D_2 receptor gene encodes two molecularly distinct isoforms, named long (D_2L, 404 bp) and short (D_2S, 317 bp). Results showed that D_1 receptor gene was distributed in the brain, with the highest density in the striatum followed by amygdala, frontal cortex and hippocampus .In case of D_2 receptor gene, the highest density was found in the striatum followed by amygdala, hippocampus and frontal cortex (Fig. 1a).

DA D_1/D_2 receptor gene expression in the frontal cortex: Exposure to CUS produced a significant decreased in the expression of D_1 receptor mRNA as compared with nonstress (NS) group ($p<0.05$). The expression of D_1 receptor mRNA was insignificantly decreased following the treatment of A 68930 (0.25 mg kg^{-1}) ($p>0.05$). However, there was no change in the expression of D_2 receptor mRNA in all the groups studied ($p>0.05$) (Fig. 2a).

DA D_1/D_2 receptor gene expression in the striatum: The D_1 receptor mRNA expression was significantly increased in AS and CUS group when compared to NS control group ($p<0.05$). Pretreatment of A 68930 (0.25 mg kg^{-1}) significantly decreased the expression of D_1 receptor gene ($p<0.05$). Expression of D_2 receptor was also significantly decreased in CUS group as compared with NS control group ($p<0.05$), but A 68930 treatment had no effect on the gene expression of D_2 receptor ($p>0.05$) (Fig. 2b).

DA D_1/D_2 receptor gene expression in the hippocampus: The D_1 receptor mRNA expression was significantly increased when rats were subjected to both AS and CUS as compared to NS control group ($p<0.05$). This increased in D_1 receptor gene expression was significantly normalized by the administration of A 68930 (0.25 mg kg^{-1}) ($p>0.05$). Whereas gene expression of D_2 receptor remains unaltered by A68930 treatment in all the groups examined (Fig. 3a).

DA D_1/D_2 receptor gene expression in the amygdala: Administration of A68930 to CUS group significantly normalized the decreased mRNA expression of D_1 receptor ($p<0.05$). Whereas, A68930 had no effect on the mRNA expression of D_2 receptor in both the stressed models ($p>0.05$) (Fig. 3b).

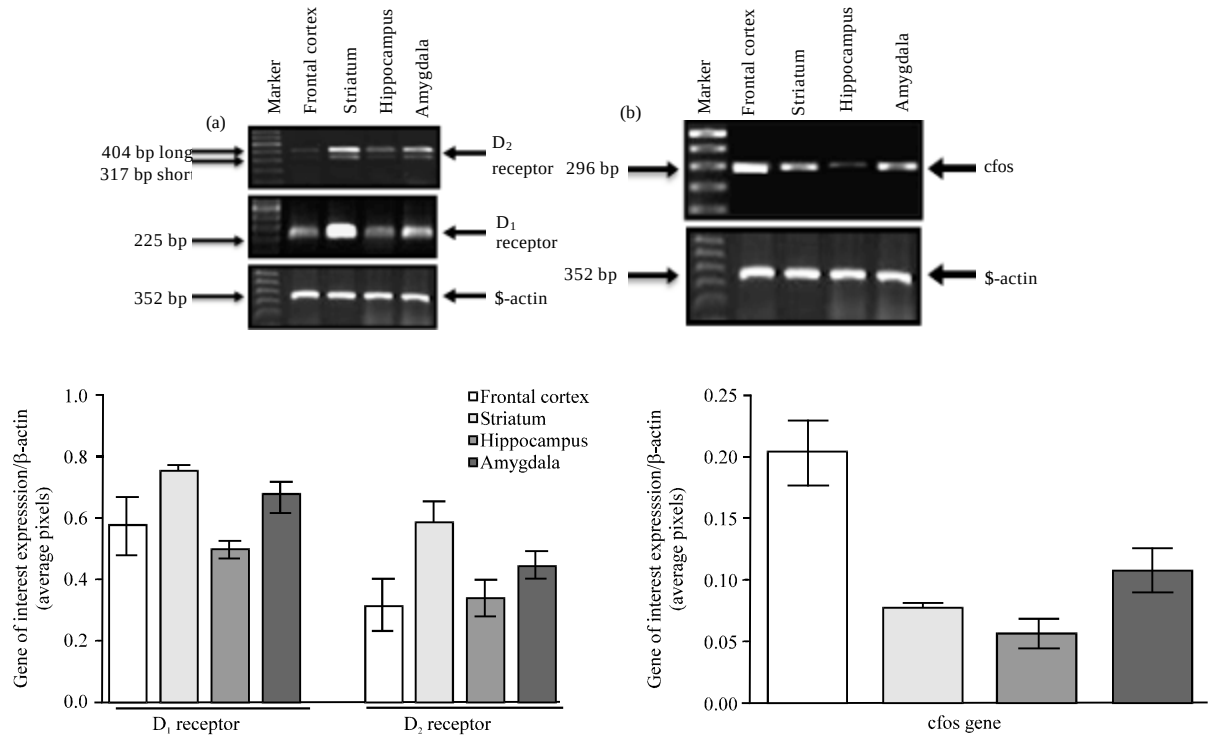

Fig. 1(a-b): Image (upper panel) and quantitative analysis result (lower panel) of the alterations in levels of gene expression of (a) D_1, D_2 receptor and (b) cfos in frontal cortex, striatum, hippocampus and amygdala were analyzed by RT-PCR. Data expressed as Mean±SEM of four independent experiments. The blots are representative and the results in the histogram are expressed as ratio of relative intensity of gene of interest to β-actin. Statistical analysis was performed by One Way ANOVA followed by Tukey-Kramer multiple comparison test

Effect of A 68930 on AS and CUS induced changes in brain cfos mRNA expression

Distribution of cfos mRNA in selected brain regions: Figure 1b shows that cfos gene was distributed in the brain, with the highest density in the frontal cortex followed by amygdala, striatum and hippocampus.

cfos gene expression in the frontal cortex and striatum: The cfos mRNA expression was significantly increased when rats were subjected to both AS and CUS as compared to NS control group in both the brain regions ($p<0.05$). Administration of A 68930 (0.25 mg kg^{-1}) had no effect on these changes ($p>0.05$) (Fig. 4a, b).

cfos gene expression in the hippocampus and amygdala: The cfos mRNA expression was significantly increased when rats were subjected to both AS and CUS as compared to NS control group in amygdala region of brain ($p<0.05$). Administration of A 68930 (0.25 mg kg^{-1}) showed no effect on this change ($p>0.05$). Whereas, in hippocampus A 68930 administration showed no effect in both AS and CUS groups ($p>0.05$) (Fig. 5a, b).

DISCUSSION

Dopamine has been implicated in the stress-related regulation of the HPA axis, as well as in depression (Zacharko and Anisman, 1991; Cabib and Puglisi-Allegra, 1996). There is evidence that central dopaminergic systems exert a positive control on the HPA axis. We observed that under baseline condition cfos gene is likely to be distributed in the brain, with the highest density in the frontal cortex followed by amygdala, striatum and hippocampus. However, the D_2 receptor gene encodes two molecularly distinct isoforms, named long (D_2L, 404 bp) and short (D_2S, 317 bp). D_1 receptor gene is distributed in the brain, with the highest density in the striatum followed by amygdala, frontal cortex and

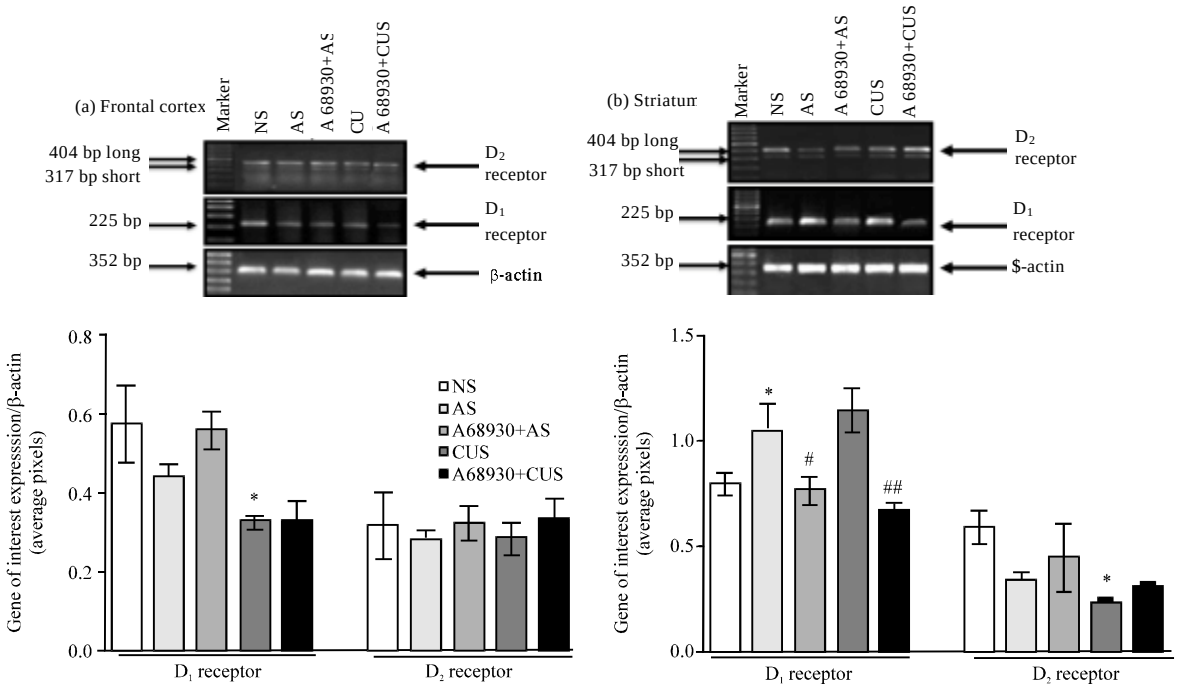

Fig. 2(a-b): Image (upper panel) and quantitative analysis result (lower panel) of the alterations in the levels of gene expression of D_1 and D_2 receptor in the (a) frontal cortex and (b) striatum in response to stress and A 68930 treatment were analyzed by RT-PCR. Data expressed as Mean±SEM of four independent experiments. The blots are representative and the results in the histogram are expressed as ratio of relative intensity of gene of interest to β-actin. Statistically significant at *$p< 0.05$, # $p<0.05$ and ##$p< 0.01$ in comparison to NS control group

hippocampus. In case of D_2 receptor gene, the highest density was found in the striatum followed by amygdala, hippocampus and frontal cortex. The particular abundance of these genes in different brain regions found in our study was well supported by others (Pacak *et al*., 1995; Fremeau *et al*., 1991; Dearry *et al*., 1990; Picetti *et al*., 1997). This differential abundance of D_1 /D_2 receptor and cfos mRNA level, suggesting their diverse role in various CNS function operated in particular brain regions.

Stress causes an induction of cfos in the frontal cortex, striatum and amygdala. An increase of cfos mRNA expression remains up regulated following the treatment of A68930 at 0.25 mg kg^{-1} dose suggested that neurons in these brain structures are activated. Interestingly, in the hippocampus significantly lesser induction of cfos suggested the different vulnerability and effect of stress on various brain regions. Present results are in agreement with other studies in which they observed a region vise activation/induction of cfos immuno-reactivity upon the exposure of various stressors (Pacak *et al*., 1995). Sustained expression of c-fos in response to long-lasting stimuli has been observed in a variety of stressful conditions. Chronic stress may cause persistent stimulation of c-fos gene expression, or neurons of certain brain nuclei or regions are activated alternatively. Whether this is due to activation of other intracellular mechanisms or to the intensity of the stimulus remains to be determined. However, induction of cfos an immediate early gene by stress in the brain regions like the frontal cortex, striatum and amygdala, further support our finding where we observed most of the dopaminergic changes in response to stress in these brain structures. Results also demonstrated that exposure of different stressful conditions (AS and CUS) produced a diverse effect on the mRNA expression of D_1 receptor, with relatively no change in D_2 receptor expression in the frontal cortex, striatum, hippocampus and amygdala. The D_1 receptor mRNA was found increased in the striatum and hippocampus both in AS and CUS groups, which was countered following the treatment of A68930 (0.25 mg kg^{-1}). On the other hand D_1 receptor mRNA level was decreased in the frontal cortex and amygdala regions in CUS group only. Most of these changes of D_1/D_2 receptor at mRNA level during stress are in line with the

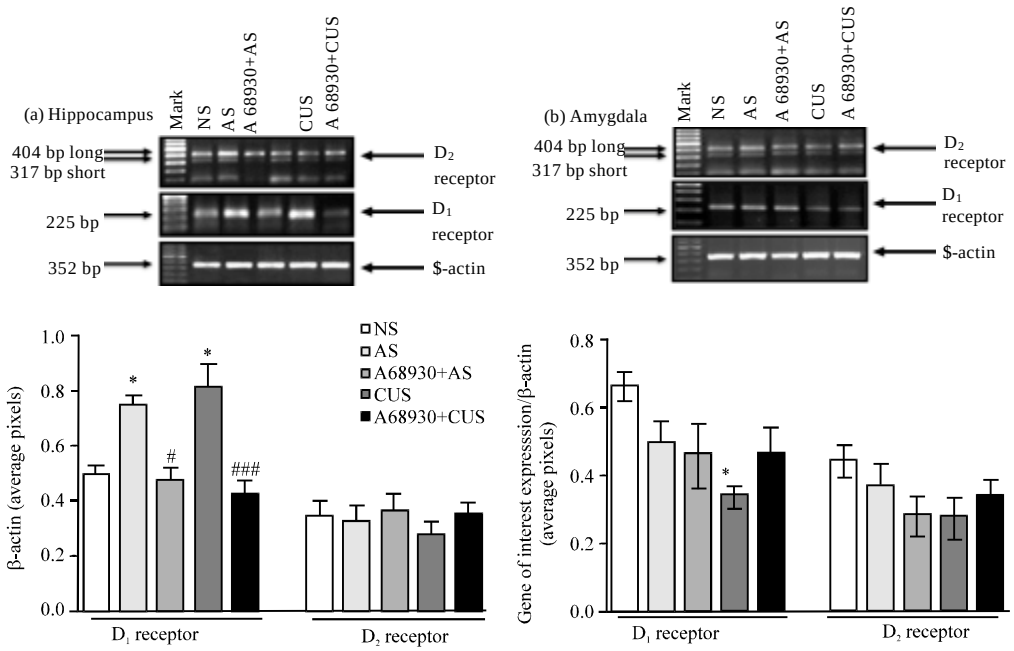

Fig. 3(a-b): Image (upper panel) and quantitative analysis result (lower panel) of the alterations in the levels of gene expression of D_1 and D_2 receptor in the (a) hippocampus and (b) amygdala in response to stress and A 68930 treatment were analyzed by RT-PCR. Data expressed as Mean±SEM of four independent experiments. The blots are representative and the results in the histogram are expressed as ratio of relative intensity of gene of interest to β-actin. Statistically significant at *$p<0.05$, # $p<0.05$ and ## $p< 0.01$ in comparison to NS control group

changes observed at the protein level. We have previously demonstrated by receptor binding studies that CUS causes a significant decrease in the number of D_1-like receptors in the frontal cortex and pretreatment with D_1 agonist A 68930 further decreased the density in this region. This could be attributed to the increase binding of D_1 receptors in presence of A 68930. However, in case of striatum and hippocampus A 68930 pretreatment ameliorates the CUS induced change in the number of D_1-like receptors. In AS, the brain regional trend of D_1-like receptor number is similar to CUS, although statistically not significant, whereas at mRNA level AS was capable of significantly up regulating D_1 receptors in the striatum and hippocampus regions of brain. The discrepancies in the expression of D_1/D_2 receptors during AS condition at the protein and gene level in discrete brain regions under stressful condition cannot be totally understood, however, insufficiency in post translational changes may be hypothesized.

Neuroanatomical studies have demonstrated that D_1 receptors are highly expressed in the striatum, nucleus accumbens and olfactory tubercle, with somewhat lower concentrations in the frontal cortex (Missale *et al*., 1998). Peripheral administration of a D_1 like receptor agonist is known to induce a robust expression of immediate early genes (e.g., c-fos) in intact habituated rats, especially in the nucleus accumbens, olfactory tubercle and cortex (Wang and McGinty, 1996). In the present study, we also found an induction of c-fos mRNA in discrete brain regions which can be correlated with the changes in motor activity. Several authors have demonstrated increased expression of D_1R binding sites in cortex, striatum, nucleus accumbens and olfactory tuberculum in rats (Papa *et al*., 2002; Kirouac and Ganguly, 1993; Watanabe *et al*., 1997), which could be a consequence of disturbances in DA uptake, storage and/or metabolism (Russell *et al*., 2005). The results may also indicate that the stimulation of dopamine D_1 receptors, possibly in the prefrontal cortex and striatum is associated with inhibitory actions on locomotor activity. These changes in D_1 like receptor density at mRNA level during AS and CUS can also be explained on the basis of corresponding changes in the levels of DA and its normalization following the treatment of A68930.

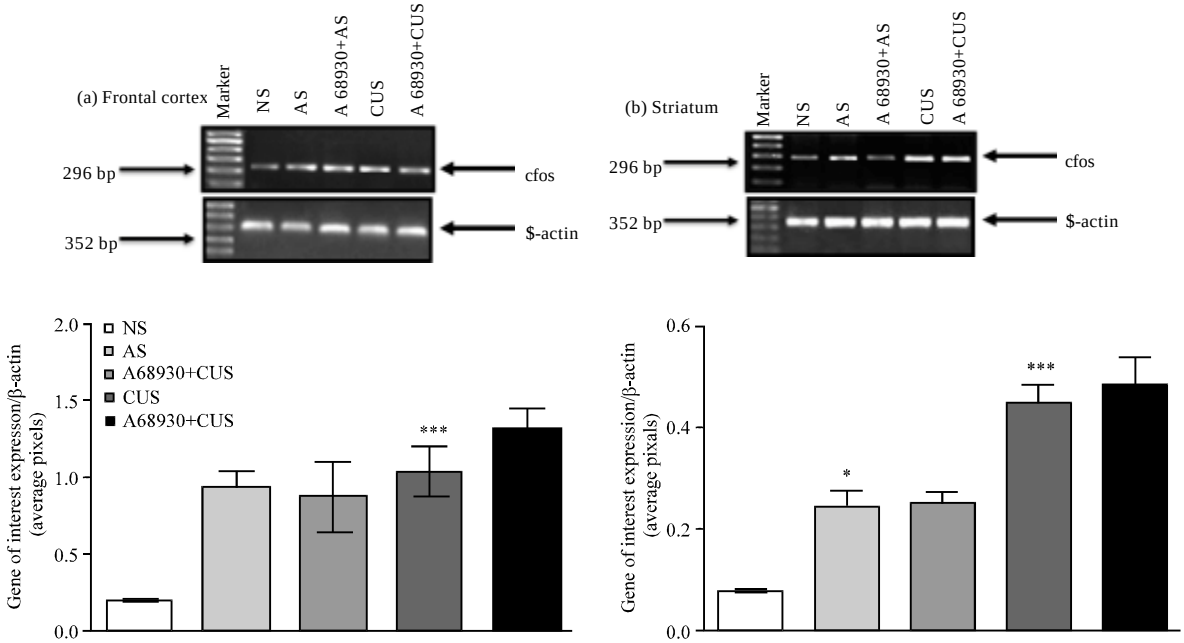

Fig. 4(a-b): Image (upper panel) and quantitative analysis result (lower panel) of the alterations in the levels of gene expression of cfos in the (a) frontal cortex and (b) striatum in response to stress and A 68930 treatment were analyzed by RT-PCR. Data expressed as Mean±SEM of four independent experiments. The blots are representative and the results in the histogram are expressed as ratio of relative intensity of gene of interest to β-actin. Statistically significant at *p< 0.05 in comparison to NS control group

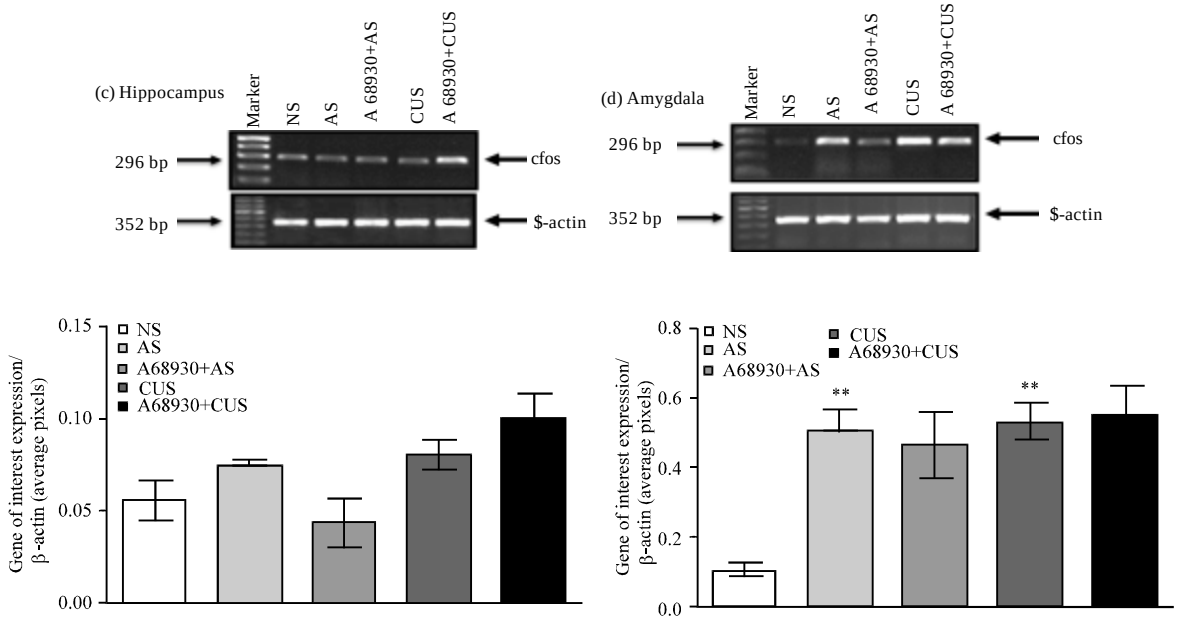

Fig. 5(a-b): Image (upper panel) and quantitative analysis result (lower panel) of the alterations in the levels of gene expression of cfos in the (a) hippocampus and (b) amygdala in response to stress and A 68930 treatment were analyzed by RT-PCR. Data expressed as Mean±SEM of four independent experiments. The blots are representative and the results in the histogram are expressed as ratio of relative intensity of gene of interest to β-actin. **p<0.05 vs NS control group

REFERENCES

Ahmad, A., N. Rasheed, N. Banu and G. Palit, 2010. Alterations in monoamine levels and oxidative systems in frontal cortex, striatum and hippocampus of the rat brain during chronic unpredictable stress. Stress, 13: 355-364.

Benturquia, N., C. Courtin, F. Noble and C. Marie-Claire, 2008. Involvement of D1 dopamine receptor in MDMA-induced locomotor activity and striatal gene expression in mice. Brain Res., 1211: 1-5.

Brunelin, J., T. d'Amato, J. van Os, A. Cochet, M.F. Suaud-Chagny and M. Saoud, 2008. Effects of acute metabolic stress on the dopaminergic and pituitary-adrenal axis activity in patients with schizophrenia, their unaffected siblings and controls. Schizophrenia Res., 100: 206-211.

Cabib, S. and S. Puglisi-Allegra, 1996. Stress, depression and the mesolimbic dopamine system. Psychopharmacology, 128: 331-342.

Dearry, A., J.A. Gingrich, P. Falardeau, R.T. Fremeau Jr., M.D. Bates and M.G. Caron, 1990. Molecular cloning and expression of the gene for a human D_1 dopamine receptor. Nature, 347: 72-76.

Fremeau Jr., R.T., G.E. Duncan, M.G. Fornaretto, A. Dearry, J.A. Gingrich, G.R. Breese and M.G. Caron, 1991. Localization of D_1 dopamine receptor mRNA in brain supports a role in cognitive, affective and neuroendocrine aspects of dopaminergic neurotransmission. Proc. Natl. Acad. Sci. USA., 88: 3772-3776.

Glowinski, J. and L.L. Iversen, 1966. Regional studies of catecholamines in the rat brain. I. The disposition of [3H] dopamine and [3H] dopa in various regions of the brain. J. Neurochem., 13: 655-669.

Hiroi, N., A.B. Martin, C. Grande, I. Alberti, A. Rivera and R. Moratalla, 2002. Molecular dissection of dopamine receptor signaling. J. Chem. Neuroanatomy, 23: 237-242.

Katz, R.J., K.A. Roth and B.J. Carroll, 1981. Acute and chronic stress effects on open field activity in the rat: Implications for a model of depression. Neurosci. Biobehav. Rev., 5: 247-251.

Kebabian, J.W. and D.B. Calne, 1979. Multiple receptors for dopamine. Nature, 277: 93-96.

Kirouac, G.J. and P.K. Ganguly, 1993. Up-regulation of dopamine receptors in the brain of the spontaneously hypertensive rat: An autoradiographic analysis. Neuroscience, 52: 135-141.

Konturek, P.C., T. Brzozowski, J. Kania, S.J. Konturek, S. Kwiecien, R. Pajdo and E.G. Hahn, 2003. Pioglitazone, a specific ligand of peroxisome proliferator-activated receptor-ã, accelerates gastric ulcer healing in rat. Eur. J. Pharmacol., 472: 213-220.

Missale, C., S.R. Nash, S.W. Robinson, M. Jaber and M.G. Caron, 1998. Dopamine receptors: From structure to function. Physiol. Rev., 78: 189-225.

Nakahara, T., M. Hirano, H. Uchimura, S. Shirali, C.R. Martin, A.B. Bonner and V.R. Preedy, 2002. Chronic alcohol feeding and its influence on c-Fos and heat shock protein-70 gene expression in different brain regions of male and female rats. Metabolism, 51: 1562-1568.

Nieoullon, A. and A. Coquerel, 2003. Dopamine: a key regulator to adapt action, emotion, motivation and cognition. Curr. Opin. Neurol., 16: S3-S9.

Pacak, K., M. Palkovits, I.J. Kopin and D.S. Goldstein, 1995. Stress-induced norepinephrine release in the hypothalamic paraventricular nucleus and pituitary-adrenocortical and sympathoadrenal activity: *In vivo* microdialysis studies. Front Neuroendocrinol., 16: 89-150.

Pani, L., A. Porcella and G.L. Gessa, 2000. The role of stress in the pathophysiology of the dopaminergic system. Mol. Psychiatry, 5: 14-21.

Papa, M., L. Diewald, M.P. Carey, F.J. Esposito, U.A.G. Carnevale and A.G. Sadile, 2002. A rostro-caudal dissociation in the dorsal and ventral striatum of the juvenile SHR suggests an anterior hypo- and a posterior hyperfunctioning mesocorticolimbic system. Behav. Brain Res., 130: 171-179.

Picetti, R., A. Saiardi, T.A. Samad, Y. Bozzi, J.H. Baik and E. Borrelli, 1997. Dopamine D2 receptors in signal transduction and behavior. Crit. Rev. Neurobiol., 11: 121-142.

Rasheed, N., A. Ahmad, C.P. Pandey, R.K. Chaturvedi, M. Lohani and G. Palit, 2010. Differential response of central dopaminergic system in acute and chronic unpredictable stress models in rats. Neurochem. Res., 35: 22-32.

Rasheed, N., A. Ahmad, M. Al-Sheeha, A. Alghasham and G. Palit, 2011. Neuroprotective and anti-stress effect of A68930 in acute and chronic unpredictable stress model in rats. Neurosci. Lett., 504: 151-155.

Russell, V.A., T. Sagvolden and E.B. Johansen, 2005. Animal models of attention-deficit hyperactivity disorder. Behav. Brain Funct. 10.1186/1744-9081-1-9

Sheikh, N., A. Ahmad, K.B. Siripurapu, V.K. Kuchibhotla, S. Singh and G. Palit, 2007. Effect of *Bacopa monniera* on stress induced changes in plasma corticosterone and brain monoamines in rats. J. Ethnopharmacol., 111: 671-676.

Van Craenenbroeck, K., K. De Bosscher, W.V. Berghe, P. Vanhoenacker and G. Haegeman, 2005. Role of glucocorticoids in dopamine-related neuropsychiatric disorders. Mol. Cell. Endocrinol., 245: 10-22.

Varga, J., A. Domokos, I. Barna, R. Jankord, G. Bagdy and D. Zelena, 2011. Lack of vasopressin does not prevent the behavioural and endocrine changes induced by chronic unpredictable stress. Brain Res. Bull., 84: 45-52.

Vrana, S.L., B.W. Kluttz and K.E. Vrana, 1995. Application of quantitative RT-PCR to the analysis of dopamine receptor mRNA levels in rat striatum. Brain Res. Mol. Brain Res., 34: 127-134.

Wang, J.Q. and J.F. McGinty, 1996. Scopolamine augments c-fos and zip/268 messenger RNA expression induced by the full D (1) dopamine receptor agonist SKF-82958 in the intact rat striatum. Neuroscience, 72: 601-616.

Watanabe, Y., M. Fujita, Y. Ito, T. Okada, H. Kusuoka and T. Nishimura, 1997. Brain dopamine transporter in spontaneously hypertensive rats. J. Nucl. Med., 38: 470-474.

Zacharko, R.M. and H. Anisman, 1991. Stressor-induced anhedonia in the mesocorticolimbic system. Neurosci. Biobehav. Rev., 15: 391-405.