Effect of haloperidol on behavioral sensitization and cognition in methylphenidate and buspirone-methylphenidate co-administered rats

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Abstract: Attenuation of methylphenidate-induced behavioral sensitization and cognitive tolerance by buspirone co-administration has been reported previously. Dopamine D_2-receptors are considered to be important in methylphenidate-induced sensitization. This study was designed to monitor the responsiveness of D_2 receptors following long-term methylphenidate, buspirone and their co-administration in rats by the challenge dose of haloperidol. Effects of haloperidol challenge dose (1 mg/kg i.p.) were monitored after 6 weeks (till the behavioral sensitization produced) from oral repeated (twice a day for 6 week) administration of methylphenidate (2mg/kg/day), buspirone (10mg/kg/day) and their co-administration. Motor activity was compared by using familiar environment of home cage and novel environment of open field and cognitive activity was compared by using water maze were monitored 30, 60, and 90 minutes post injection respectively. We found that haloperidol reduced motor activity in familiar as well as in novel environment and showed impaired cognitive performance in water maze. The effects were more pronounced in methylphenidate treated rats as compared to buspirone and methylphenidate co-administration treated rats. Increased response of haloperidol in methylphenidate treated rats can be explained in terms of super-sensitization of D_2 receptors, which results in behavioral sensitization that is not observed in co-administration treated rats. Buspirone prevents D_2 receptor's super-sensitization by increasing serotonergic inhibitory influence on dopamine neuron.

Keywords: Methylphenidate, Buspirone, cognition, sensitization, D_2 receptors, Haloperidol.

INTRODUCTION

Central nervous system stimulants are the drugs that produce euphoria, increase intellect, cognition, performance and delay onset of fatigue. Despite these beneficial effects, most central nervous system stimulants are of little therapeutic value because long-term use of these drugs produces tolerance on cognitive behavior, reverse tolerance on motor activity and dependence (Leith and Kuczenski., 1982). These drugs are, therefore, classified as the drugs of abuse (Robinson and Berridge, 2008).

Methylphenidate, a drug of choice in attention deficit hyperactivity (ADHD), causes amplification of dopamine (DA) release in the central nervous system that contributes to the attention enhancing aspect of the drug (Volkow *et al*., 2002). It blocks noradrenalin and DA transporter protein (Barrett *et al.*, 2005) and enhances extra-cellular concentrations of catecholamine**.** Therapeutic doses of methylphenidate that improve cognition in rats increases norepinephrine and DA efflux in Prefrontal cortex (PFC) relative to limbic structures (Berridge *et al*., 2006). DA reuptake in PFC is through both the norepinephrine transporter and the DA transporter (Sesack *et al*., 1998; Yamamoto and Novotney, 1998). It is likely that the sensitization-inducing effects and therapeutic efficacy of methylphenidate are connected to modification in DA and norepinephrine transmission in PFC. The brain dopaminergic system and the involvement of DA D_2-receptors are considered to be important in methylphenidate-induced sensitization (Millan *et al*., 1998). Sensitization to amphetamine and methamphetamine was reported to be prevented by D_2 antagonist (Meng *et al*., 1998; White *et al*., 1998).

Drugs of abuse after long term administration enhance the effectiveness of 5-HT_{1A} somatodendritic receptors. Cognitive tolerance and motor sensitization can be modulated by the central serotonergic system (Hall *et al*., 2004; Muller *et al*., 2003). Repeated buspirone administration attenuates psychostimulant-induced behavioral sensitization and cognitive tolerance (Alam *et al*., 2015; Alam *et al*., 2016).

Haloperidol, a dopamine D_2 receptor antagonist, known to block the effect of amphetamine on motor cortex (Feeney and Hovda, 1983) and reduce motor function in rats (Feeney *et al*., 1982). Haloperidol, when injected into the rat nucleus accumbens, disturbs acquisition antagonism that may affect cognitive outcome. Studies show that Morris water maze acquisition in normal rats is disturbed after chronic haloperidol treatment (Terry *et al*., 2002).

The present study is designed to monitor the responsiveness of D_2 receptors by the challenge dose of haloperidol, following long-term oral administration of methylphenidate, buspirone and their co-administration

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and to investigate whether co-administration of buspirone increases the usefulness and reduces the side effects of methylphenidate.

MATERIALS AND METHODS

Animals

Forty eight rats were caged in pairs, in a quiet room, under a 12 hr. cycle of light/dark and room temperature was controlled at 24±2°C. Cubes of standard rodent diet with free tap water access was given so that rats could become familiar to the environment. They were acclimatized to various handling procedures in order to nullify stress effects. All the performed experiments were according to the approved protocols and were in accordance with the rules and regulations given by local animal care committee.

Drugs

Methylphenidate HCl purchased from Sigma was dissolved in saline NaCl 0.9% and buspirone (Research Biochemicals Incorporated) was prepared in distilled water. The drugs were administered by oral route twice a day individually and also co-administered to the 3rd group. Methylphenidate at the dose of 2mg/kg/day (Aoyama *et al.,* 1990), buspirone at the dose of 10mg/kg/day (Naito *et al.,* 2003) and co-administered methylphenidate at the dose of 2mg/kg/day and buspirone at the dose of 10mg/kg/day according to the weight of the rats whereas, the control rats were treated with (0.9%) saline per oral twice a day. Haloperidol available in 5 mg/ml ampoules manufactured under license from G.D. Searle and Co. U.S.A, by Searle Pakistan Ltd. Laboratories was used in the present study.

Experimental design

The protocol of experiment was designed to administer methylphenidate and saline to 1st group of treated rats, buspirone and saline to 2nd group of treated rats, methylphenidate and buspirone to 3rd group of treated rats and saline to control rats orally two times daily (8.00 AM and 8.00 PM) for six weeks till the sensitization is produced. Among the three groups of treated rats, the 1st group was given methylphenidate in the dose of 2mg/kg/day, the 2nd group was given buspirone at the dose of 10mg/kg/day and 3rd group was given methylphenidate at the dose of 2mg/kg/day and buspirone at the dose of 10mg/kg/day according to the weight of the rats. Controlled rats were given saline 2.0ml/kg/day according to the weight of the rats. After six weeks challenge, the dose of Haloperidol 1mg/kg i.p. was given to all the treated and controlled rats to monitor the drug effects on the behavioral parameters 30 minutes post injection.

Behavioral studies

To avoid the order effect all the treatments were carried out in a balance design. The behavioral parameters monitored during treatment are as follows.

Home cage activity (familiar environment)

To monitor activity of rats in familiar environment, home cages were used. Cages were (26x26x26 cm) transparent Perspex with sawdust cover floor. In these cages, the rats were placed individually for 15 min before monitoring the activity to make them familiar with the environment. The numbers of cage-crossings after 15 minutes were counted for 10 minutes (Batool *et al*., 2000). The activity of methylphenidate, buspirone, co-administration and saline treated rats in the familiar environment were monitored 30 minutes post haloperidol injection. The activities of the controlled and the test animals were monitored in a balanced design to avoid order effect.

Open field activity (novel environment)

The novel environment of open field consists of a square area (76x76 cm) with 42 cm high walls and the apparatus floor was divided by lines into 25 squares all of the equal size. The experiment was performed under the white light in a quiet room. The animal taken out from the cage was placed in the open field at the center square. The numbers of squares crossed were counted for 5 minutes (Ikram *et al*., 2007). Activity of the drug treated groups and the controlled rats, were monitored 60 minutes post haloperidol injection. To avoid the order effect, the open field activity of the rats was scored in a balanced design.

Water Maze test

In the present study, the Water Maze apparatus used, consisted of a (60x30cms) transparent glass tank, rectangular in shape, filled with room temperature-water opacified with powdered milk, to the depth of 12cm. A wooden platform (15x13cms) was placed at a fixed location, hidden 2cm below the surface of the water. The effects on the spatial memory were examined by assessing performance in Water Maze (WM) test. Initially and during the training sessions, the rats were trained, every rat was placed into the tank of water facing the wall and allowed 2 min to climb and locate onto the submerged platform. Within the time allowed, if the rat failed to find the platform, it was guided gently onto the platform. After the acquisition phase, retention latency was recorded to test the memory function of the rats. The retention latency is the time taken by each rat to locate the hidden platform after the training. The cognitive test of the drug treated and the controlled animals were monitored 90 minutes post haloperidol injection. The experiment was performed in a balanced design to avoid the time and the order effect.

STATISTICAL ANALYSIS

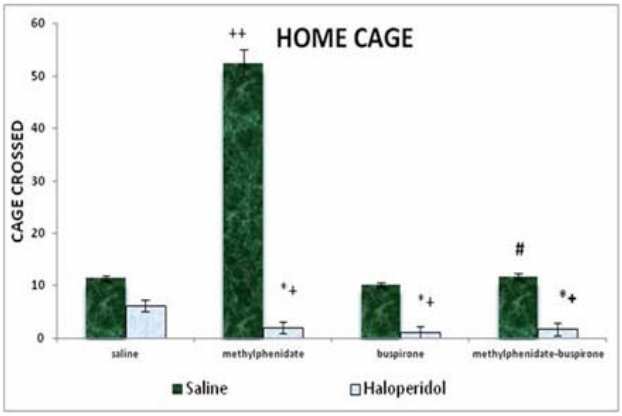
The results are represented as mean ±S.D. The statistical analysis was performed by using the SPSS software (version 16.0). The data on the effects of haloperidol on the drug treated and the controlled animals in home cage, open field and water maze were performed using three-

way ANOVA analysis of variance. Newman-Keul test was performed for Post hoc comparison of the groups. The level of significance was considered significant if the values lied between $p<0.05$ and $p<0.01$.

RESULTS

Effect of Haloperidol on motor activity in familiar environment in Methylphenidate treated, Buspirone treated and co-administration of Methylphenidate-Buspirone treated rats.

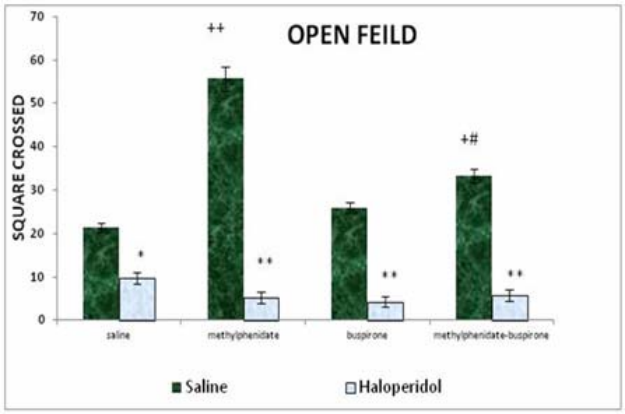
The data on home cage analyzed by three-way ANOVA showed significant effects of methylphenidate ($F=112.2$, $df=1,24$, $p<0.01$), buspirone ($F=57.4$, $df=1,24$, $p<0.01$) haloperidol ($F=241$, $df=1,24$, $p<0.01$) and significant interactions between haloperidol*buspirone ($F=36.9$, $df=1,24$, $p<0.01$), haloperidol*methylphenidate ($F=74.9$, $df=1,24$, $p<0.01$), buspirone*methylphenidate ($F=116.8$, $df=1,24$, $p<0.01$) and haloperidol*buspirone *methylphenidate ($F=107.7$, $df=1,24$, $p<0.01$).



The effect of haloperidol 1mg/kg I.P. on familiar environment (home cage) in methylphenidate treated, buspirone treated and co-administration of methylphenidate-buspirone treated rats. The values are means ±SD (n=6). The significant differences by Newman-Keuls test:*$p<0.01$ from similarly treated saline injected rats. ++$p<0.01$,+$p<0.05$ from similarly injected saline treated rats. #$p<0.01$ from similarly injected methylphenidate treated rats following three-way ANOVA.

Fig. 1: Effect of haloperidol in methylphenidate, buspirone and their co-administration treated rats on familiar environment.

Post hoc Newman–Keuls test demonstrated that the activity of methylphenidate treated saline injected rats was greater ($p<0.01$) in a familiar environment than saline treated saline injected rats. Haloperidol decreased the cage crossing in methylphenidate, buspirone and methylphenidate-buspirone co-administered rats significantly ($p<0.01$) compared to the similarly treated saline injected rats and significantly ($p<0.05$) from the similarly injected saline treated rats. Buspirone significantly decreased ($p<0.01$) the cage crossings in co-administration treated and saline injected rats but revealed no effect on haloperidol injected rats than similarly injected methylphenidate treated rats.



The effect of Haloperidol 1mg/kg I.P. on square crossing of novel environment (open field) in methylphenidate treated, buspirone treated and co-administration of methylphenidate-buspirone treated rats. The values are means ±SD (n=6). The significant differences by Newman–Keuls test:*$p<0.05$, **$p<0.01$ from similarly treated saline injected rats. +$p<0.05$, ++$p<0.01$ from similarly injected saline treated rats. #$p<0.01$ from similarly injected methylphenidate treated rats following three-way ANOVA.

Fig. 2: Effect of haloperidol in methylphenidate, buspirone and their co-administration treated rats on novel environment.

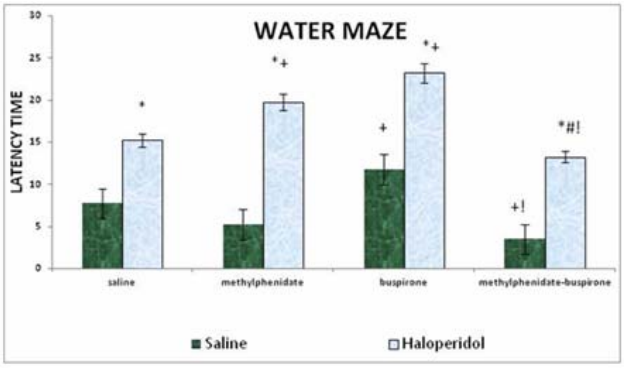
Effect of Haloperidol on motor activity in novel environment in Methylphenidate treated, Buspirone treated and co-administration of Methylphenidate-Buspirone treated rats.

The data analyzed by three-way ANOVA showed significant effects of methylphenidate ($F=39.4$, $df=1,24$, $p<0.01$), haloperidol ($F=160.8$, $df=1,24$, $p<0.01$) and non-significant effects of buspirone ($F=3.8$, $df=1,24$, $p>0.05$). The interactions between haloperidol*buspirone ($F=6.2$, $df=1,24$, $p<0.05$), haloperidol *methylphenidate ($F=14.2$, $df=1,24$, $p<0.01$), buspirone *methylphenidate ($F=5.9$, $df=1,24$, $p<0.05$) and haloperidol*buspirone *methylphenidate ($F=17.7$, $df=1,24$, $p<0.01$) are significant.

Post hoc Newman–Keuls test demonstrated that haloperidol significantly ($p<0.01$) decreased square crossed activity in methylphenidate, buspirone and co-administration treated rats and ($p<0.05$) in saline treated rats from similarly treated saline injected rats. Square crossed activity increased significantly ($p<0.01$) in saline injected methylphenidate treated and significantly increased ($p<0.05$) in saline injected co-administration treated rats from the similarly injected saline treated rats whereas significantly decreased ($p<0.01$) in saline injected co-administration treated rats from similarly injected methylphenidate treated rats.

Effect of Haloperidol on memory in Methylphenidate treated, Buspirone treated and co-administration of Methylphenidate-Buspirone treated rats

The data analyzed by three-way ANOVA showed significant effects of methylphenidate ($F=12.22$, $df=1,24$, $p<0.01$), haloperidol ($F=63.26$, $df=1,24$, $p<0.01$) and non-significant effect of buspirone ($F=0.12$, $df=1,24$, $p>0.05$). The interactions between haloperidol*buspirone ($F=0.22$, $df=1, 24$, $p>0.05$), haloperidol *methylphenidate ($F=0.47$, $df=1,24$, $p>0.05$), haloperidol*buspirone *methylphenidate ($F=3.99$, $df=1,24$, $p>0.05$) are non-significant and the significant interaction between buspirone *methylphenidate ($F=18.15$, $df=1,24$, $p<0.01$).



The effect of Haloperidol 1m/kg I.P. on Water Maze in methylphenidate treated, buspirone treated and co-administration of methylphenidate-buspirone treated rats. The values are means ±SD (n=6). The significant differences by Newman-Keuls test: *p<0.01 from similarly treated saline injected rats. +p<0.01 from similarly injected saline treated rats. #p<0.01 from similarly injected methylphenidate treated rats. !p<0.01 from similarly injected buspirone treated rats following three-way ANOVA.

Fig. 3: Effect of haloperidol in methylphenidate, buspirone and their co-administration treated rats on memory

Post hoc analysis by Newman–Keuls test demonstrated that haloperidol significantly decreased ($p<0.01$) memory in saline, methylphenidate, buspirone and co-administration of methylphenidate-buspirone treated rats from saline injected similarly treated rats. Memory significantly ($p<0.01$) enhanced in saline injected co-administration treated rats but significantly decreased ($p<0.01$) in saline and haloperidol injected buspirone treated and haloperidol injected methylphenidate treated rats from the similarly injected saline treated rats. Significant memory ($p<0.01$) improvement occur in saline and haloperidol injected co-administration treated rats from similarly injected buspirone treated rats and in haloperidol injected co-administration treated rats from similarly injected methylphenidate treated rats.

DISCUSSION

It has been reported previously that buspirone can attenuate methylphenidate-induced behavioral sensitization and cognitive tolerance (Alam *et al.,* 2015; Alam *et al.,* 2016). In the present study, the responsiveness of D_2 receptors were studied following long-term methylphenidate, buspirone and their co-administration in rats by the haloperidol challenge dose. Haloperidol belongs to the class of neuroleptics that blocks postsynaptic D_2 receptors, affect motor and cognitive functions in rats (Terry *et al*., 2002; Feeney *et al*., 1982). Challenge dose of haloperidol reduced motor activity in familiar environment of home cage and novel environment of open field (figs. 1 and 2). The activity of rats in all treated and control groups were greater in open field as compare to the home cage, which could be explained in terms of anxiogenic effect of novel environment of open field. Indeed other authors also have reported anxiety and increase locomotor activity by using various paradigms (Bina *et al.,* 2014). The decrease in motor activity (fig. 1 & 2) and cognitive performance (fig. 3) were greater in all the drug treated groups of rats in comparison to controlled rats but among similarly treated groups, the effect of haloperidol was significantly higher in methylphenidate treated rats as compared to the buspirone treated and co-administration treated groups. Various studies documented that haloperidol has been used to assess the sensitized behavioral responses produced by the administration of psychostimulants (Costa *et al.,* 2004; Hatzipetros *et al.,* 2007)

Stimulants are supposed to be harmless ways for enhancing energy and concentration levels, increasing school performance or using for recreation and reducing the desire and duration of sleep (Habibzadeh *et al.,* 2011). Youths show an increasing predilection for the consumption of prescription stimulants (Johnston *et al.,* 2007). Previous studies have demonstrated that the repeated methylphenidate administration can cause behavioral sensitization and cognitive tolerance (Alam and Najam, 2013; Alam and Najam, 2014; Juárez and Vázquez-Cortés, 2010). The cognitive effects of methylphenidate can be task dependent on the brain function (Alam and Najam, 2014; Volkow and Swanson, 2008; Clatworthy *et al.,* 2009). For example, methylphenidate administration leads to decrease activation during decision making (Schlösser *et al.,* 2009) and reversal learning (Dodds *et al.,* 2008) tasks, but during reaction time tasks, there is an increased activation (Müller *et al.,* 2005).

The brain dopaminergic system and the involvement of DA D_2-receptors are considered to be important in methylphenidate-induced sensitization and cognitive tolerance (Millan *et al.,* 1998). DA modulates cognitive performance in part via its regulation of the prefrontal cortex through DA D_1 and D_2 receptors (Goldman-Rakic, 1998). Methylphenidate increases DA signaling in the brain and is used in the treatment of ADHD and other neuropsychiatric disorders to enhance attention and

cognition (Ackerman and White, 1992; Clatworthy *et al*., 2009). Sensitization to amphetamine and methamphetamine was reported to be prevented by D_2 antagonist (Meng *et al.,* 1998; White *et al.,* 1998). Psychostimulant-induced behavioral sensitization (Juárez and Vázquez-Cortés, 2010) is directly related with subsensitization of DA D_2 auto receptor (Bevan, 1983) and supersensitization of DA postsynaptic receptors (Ackerman and White, 1992, Hopf *et al.,* 2007). In the present study methylphenidate increase locomotor activity monitored in familiar and novel environments, whereas methylphenidate and co-administration of methylphenidate and buspirone produce cognitive enhancement monitored in Water Maze, however challenge dose of haloperidol reduce motor activity and impaired cognitive performance. Acute haloperidol administration produces the behavioral suppressant effects by blocking postsynaptic D_2 receptors (Klemm, 1993). Haloperidol is known to block the effect of amphetamine produced on motor cortex (Feeney and Hovda, 1983) and slowed motor function in rats (Feeney *et al.,* 1982). Morris Water Maze acquisition also shown to disturbed after haloperidol treatment in normal rats (Terry *et al.,* 2002**).** The effect of haloperidol on locomotor activity and cognitive performance were greater in methylphenidate treated rats compared to saline and co-administration treated rats. In the regulation of behavior central serotonergic system also play an important role along with dopaminergic system (Oades, 2008). Serotonin has inhibitory influence on the activity of DA neurotransmitter (Haleem, 2006). Therefore psychostimulant-induced behavioral sensitization could be modulated by serotonergic system (Hall *et al.,* 2004; Muller *et al.,* 2003).

Psychostimulant induced sensitization increases 5HT, hyperactivates $5HT_{1A}$ receptors, which in addition to DA is a core mechanism of action for the drug addiction. $5HT_{1A}$ receptors mainly facilitate psychostimulant addiction related behavior (Muller *et al.,* 2007). Drugs of abuse after long term administration enhance the effectiveness of $5\text{-}HT_{1A}$ somatodendritic receptors. Repeated Buspirone administration, decreased somatodendritic $5\text{-}HT_{1A}$ receptor responsiveness (Haleem *et al.,* 2007). Somatodendritic $5\text{-}HT_{1A}$ receptors desensitization by buspirone co-administration will increase the serotonergic inhibitory influence on the effect of DA neurons (Khan and Haleem, 2006) to reduce cognitive tolerance and locomotor sensitization produced by psycho-stimulants. It has been observed that there is significant reduced motor activity and cognitive performance in all treated groups but effect was more pronounced in methylphenidate treated rats after haloperidol challenge dose. From this it can be suggested that observed effect may be due to increased D_2 receptor sensitivity following long-term methylphenidate administration leads to behavioral sensitization and cognitive tolerance that could be prevented by buspirone co-administration.

CONCLUSION

In conclusion the increased response of haloperidol in methylphenidate treated rats can be explained in terms of super-sensitization of D_2 receptors that is not observed in co-administration treated rats. It is suggested that co-administration of methylphenidate and buspirone prevents D_2 receptors super-sensitization by increasing serotonergic inhibitory influence on the DA neurons and facilitate the use of methylphenidate in different cognitive disorders.

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