Attenuation of methylphenidate-induced sensitization by co-administration of buspirone

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Abstract: Methylphenidate, which inhibit dopamine transporter is effective in the treatment of ADHD (attention deficit hyperactivity disorder), but long term use of this drug is often associated with addiction and dependence. Locomotor sensitization development to psychostimulants like methylphenidate is an important contributor to drug abuse induced by psychostimulants. Different studies have shown that long term administration of drugs of abuse increases the effectiveness of 5-hydroxytryptamine (5-HT) 1A somatodendritic receptors. Repeated buspirone administration reduces the effectiveness of 5-HT 1A somatodendritic receptors. This study was designed to determine that buspirone co-administration may reduce methylphenidate-induced sensitization. The motor activity was compared by using familiar and novel environments after long-term administration of methylphenidate, buspirone and their co-administration. Long term oral administration of methylphenidate at a dose of 2.0mg/kg/day enhanced motor activity in home cage i.e activity of familiar environment monitored at alternate day. Locomotor enhancing effects of methylphenidate were augmented on 13th day of drug administration suggesting sensitization induced by the drug. The sensitization effects were significant in home cage monitored on alternate day and also in an open field monitored weekly. Buspirone co-administration at a dose of 10mg/kg/day prevented methylphenidate-induced sensitization. It is suggested that the sensitization development to methylphenidate may oppose by buspirone co-administration due to the reduction in the sensitivity of 5-HT 1A somatodendritic receptors. These findings may help extend future therapeutics in ADHD.

Keywords: Methylphenidate, buspirone, open field, home cage and sensitization.

INTRODUCTION

Psycho-stimulants like methylphenidate have long been utilized in young children with ADHD (attention-deficit hyperactivity disorder) (Pliszka., 2007, Greendhill et al., 2002). ADHD is identified as hyperactivity, inattention and impulsivity disorder. Patients identified with ADHD display noradrenergic and dopaminergic dysfunction within caudate nucleus (CN) and prefrontal cortex (PFC) (Arnsten and Dudley, 2005, Bush et al., 2005, Sergeant et al., 2002, Seidman et al., 2004). Investigations in children with ADHD have shown influences on self-esteem, academic attainment, employment stability, social and professional functioning (Barkley et al., 1990). The principle objectives of treatment are not simply to treat the symptoms but in addition to restore optimal functionality (Steele et al., 2006).

Despite of different beneficial effects, psychostimulants are also categorized as the drug of abuse because of their dependence and sensitization (Berridge and Devilbiss 2011., Solanto. 1998, Segal. 1975, Steketee, 2005). With repeated exposure to particular drug, the augmented motor-stimulant responses is characterized by behavioral sensitization (Steketee and Kalivas., 2011). Development of locomotor sensitization to psycho-stimulant drug is an important predictor of psycho-stimulant drug abuse in animal models (Robinson and Berridge., 1993).

Methylphenidate enhances levels of dopamine in the neocortex (Berridge et al., 2006). It blocks the dopamine transporter (Ferris and Tang., 1979, Ritz et al., 1987, Kolls et al., 2001, Barrett et al., 2005) thus enhances extra cellular concentration of dopamine and this action is thought to be the initiating molecular event that reinforces drug seeking behavior, eventually culminating in addiction (Teter, et al., 2006, Alizadeh and Ghabili. 2008). Acute intake of methylphenidate creates hyperactivity in rodents which is further enhanced with its repeated administration (Castellanos and Tannock. 2002, Rubia et al., 2010, Schecklmann et al., 2010).

Studies have shown that the dopamine system is very important for development and expression of behavioral sensitization (Kalivas et al., 1993a, 1993b). It has been shown that dopamine release was directly correlated with the consumption history of psycho stimulant, suggesting an increased response of dopamine with repeated use of drug (Cox et al., 2009). The stimulation of D2 dopamine receptor is crucial for the diverse physiological functions regulation, like locomotor activity control (Picetti et al., 1997). Serotonin has inhibitory influence on dopamine neurotransmission in the mid brain as well as in the terminal region (Haleem, 2006).

Buspirone is agonist at 5-hydroxytryptamine (5-HT) 1A auto receptors and an antagonist at certain postsynaptic 5HT 1A receptor site.5-HT 1A somatodendritic inhibitory
receptors that control release of 5-HT are readily desensitized by chronic stimulation with a high-efficacy 5-HT1A agonist. 5-HT1A auto receptors desensitization leads to an increased 5-HT release which results in tonic activation of 5-HT1A postsynaptic receptors in the hippocampus and 5-HT2C in dopaminergic neuron (Haddjeri et al., 1998). Increased 5-HT2C expression decreases dopamine release and 5-HT2C receptor antagonism results in an increased extracellular dopaminergic response to many drugs (Bubaret al., 2006, Esposito et al., 2006).

Previously it is reported that repeated administration of oral therapeutic doses of methylphenidate produces locomotor sensitization whereas sensitization and cognitive improvement are more pronounced at smaller doses (Alam and Najam., 2013). The purpose of this study was to investigate the role of 5-HT1A somatodendritic receptors in behavioral sensitization produced after repeated administration of methylphenidate (Bloise et al., 2007, Haleem and Khan., 2003). It was hypothesized that 5-HT1A somatodendritic receptors desensitization by buspirone co-administration will increase the serotonin inhibitory influence on dopamine neurons to reduce the locomotor sensitization already reported for methylphenidate (Khan and Haleem., 2006). This study was designed to determine the effects of buspirone co-administration on motor sensitization as induced by repeated methylphenidate administration.

MATERIALS AND METHOD

Animals
Albino Wistar rats bred locally, weighing 180-200g, were individually housed under 12h dark and light cycles and controlled room temperature at 24±2°C. Animals were given access to free tap water and rodent diet cubes at least 7 days before starting the experiment to familiarize them with the environment. To eliminate the effects of stress, the rats were accustomed to different handling procedures. All the experiments were performed according to the approved protocols and were in accordance to the rules and regulation given by local animal care committee.

Behavioral parameters
Activity in a familiar environment of home cage. Home cages were used to monitor the activity of rats in familiar environment. Cages are (26x26x26cm) transparent perspex with sawdust cover floor. Rats were individually placed in these cages for 15min before monitoring the activity to get familiar with the environment (Batool et al., 2000). After 15minutes the numbers of cage-crossings were counted for 10 minutes.

Activity in a novel environment of open field
Open field consists of square area (76x76cm) with 42cm high walls. The floor of open field is divided into 25 squares of equal size. Experiment was performed under white light in a quiet room. Animals were placed in the open field at the centre square. Numbers of square crossed by all four paws and latency to move were counted for 5 minutes (Ikram et al., 2007).

Drugs
Methylphenidate HClwas obtained from local medical store and prepared in 0.9% NaCl (saline) and buspirone (Research Biochemicals Incorporated) prepared in distilled water. Drugs were administered in a volume of 1 ml/kg of body weight by per oral route twice a day individually and also co-administered to the 3rd group of treated animals. Control animals were treated with saline (0.9%) at the dose of 1 ml/kg PO twice a day.

Experimental protocol
The protocol of experiment was designed to administer methylphenidate to 1st group of treated animals, buspirone and saline to 2nd group of treated animals, methylphenidate and buspirone to 3rd group of treated animals and saline to control animals orally two times daily (8.00 AM and 8.00 PM) for 6 weeks. Among the three groups of treated rats 1st group were given methylphenidate at the dose of 2mg/kg/day, 2nd group were given buspirone at the dose of 10mg/kg/day and 3rd group were given methylphenidate at the dose of 2mg/kg/day co-administered with buspirone at the dose of 10mg/kg/day according to the weight of the rats. Activity of drug treated and control rats in familiar environment were monitored on every alternate day during six weeks where as in novel environment were monitored on 1st day and weekly during the six weeks. To avoid order effect the experiment was performed in a balance design.

STATISTICAL ANALYSIS

Results are represented as mean ±S.D. Statistical analysis was performed by using SPSS software (version 16.0). Data on the effect of methylphenidate, buspirone and co-administration of methylphenidate-buspirone on activity at alternate day in familiar environment and weekly in novel environment were statistically tested by three-way (ANOVA) analysis of variance repeated measure design to see the effects of various factors involved. Newman-Keuls test was performed for post hoc comparison and P<0.01 and P<0.05 were considered as significant values.

RESULTS

Effect of repeated administration of methylphenidate, buspirone and their co administration on locomotor activity in home cage (familiar environment)
Fig. 1 shows effects of repeated methylphenidate, buspirone and their co-administration on locomotor activity in home cage (activity box) on alternate days for 6 weeks. Analysis of data by 3-way ANOVA repeated
measure design showed significant effects of methylphenidate (df=1.19, F=19.48, p<0.01), buspirone (df=1.19, F=47.43, p<0.01) and repeated monitoring (df=2.40, F=18.71, p<0.01). Interactions between buspirone* methylphenidate (df=1.19, F=3.8, p<0.01), day*buspirone (df=3.17, F=79.5, p<0.01), day*buspirone*methylphenidate (df=5.15, F=70.9, p<0.01) and day*methylphenidate (df=3.17, F=93.9, p<0.01) were significant.

**Effect of methylphenidate, buspirone and their co-administration on motor activity in familiar environment**

Fig- 1: Effect of methylphenidate, buspirone and their co-administration on motor activity in familiar environment of home cage (from day 1 to day 41 at alternate day). Values are means ± SD (n=8). Significant differences by Newman-Keuls test: *p<0.01 from similar week saline treated animals; +p<0.01 from similar week methylphenidate treated animals; #p<0.01 from similarly treated first day values following three-way ANOVA (repeated measure design).

Newman-Keul test showed that methylphenidate significantly increased activity in a home cage upon repeated administration from 13th day till 41st day (p<0.01) as compared to saline treated controls and from similarly treated first day values. Buspirone and co-administration (methylphenidate + buspirone) in familiar environment did not alter activity as compared to saline treated controls. Buspirone attenuated methylphenidate induced increase in activity was more pronounced (P<0.01) from 21st day till 41st day as compared to methylphenidate treated rats.

**Effect of repeated administration of methylphenidate, buspirone and their co-administration on motor activity in novel environment**

Fig- 2: Effect of methylphenidate, buspirone and their co-administration on square crossing in novel environment of open field (from day 1 to 6th week). Values are means ± SD (n=8). Significant differences by Newman-Keuls test: *p<0.05, **p<0.01 from similar week saline treated animals; #p<0.01 from similarly treated first day values; +p<0.01 from similar week methylphenidate treated animals; !p<0.01 from similar week buspirone treated animals following three-way ANOVA (repeated measure design).

Newman-Keul test showed that from 2nd till 6th week methylphenidate administration increased motor activity significantly (P<0.01) as compared to saline treated controls and from their first day values. Buspirone administration significantly (P<0.01) decreased activity from 2nd till 6th week as compare to the first day values and decreased significantly (P<0.05) in 3rd and 4th week as compared to saline treated controls. In co-administration treated rats motor activity significantly (P<0.01) decreased as compared to similar week methylphenidate treated rats from 2nd till 6th week whereas significantly increased as compare to similar week buspirone treated rats (P<0.01) and saline treated controls (P<0.05).

**DISCUSSION**

The development of locomotor sensitization to psycho stimulants like methylphenidate is an important contributor to drug abuse induced by psycho stimulants (Robinson and Berridge., 1993). In the present study oral therapeutic dose (2.0mg/kg/day) of methylphenidate was administered to determine the locomotor sensitization
development and reduction of methylphenidate-induced sensitization by oral co-administration of buspirone (10mg/kg/day) via monitoring the rat’s activity in the home cage i.e. familiar environment, and in the open field i.e. novel environment. Locomotor activity in home cage was monitored on every alternate day and in open field to maintain environment novelty, activity was monitored on day 1 and then weekly because monitoring frequent activity in open field could result in familiarization. Administration of methylphenidate produced locomotor sensitization in both familiar and novel environments i.e. on 13rd day of drug administration in the home cage and in 2nd week in the open field i.e. novel environment where as buspirone administration decreased activity after 2 weeks in both environments and could attenuate methylphenidate-induced hyper locomotion in familiar and novel environments following co-administration. The activity of rats treated with both the drugs i.e. methylphenidate and buspirone have shown almost constantly same activity throughout 6 weeks of drug administration.

Methylphenidate used in children with ADHD (Pliszka, 2007, Greenhill et al., 2002) enhances extracellular concentrations of dopamine by blocking the dopamine transporter (Ferris and Tang, 1979, Ritz et al., 1987, Kollins et al., 2001, Barrett et al., 2005). This action is thought to be the initiating molecular event that reinforces drug-seeking behaviors, eventually culminating in addiction (Teter, et al., 2006, Alizadeh and Ghabili, 2008).

Role of dopamine is important in the psychostimulant-induced addiction as well as increase in motor activity (Robinson and Berridge, 2000). Subsensitization of dopamine D2 auto receptor (Bevan, 1983) and super sensitization of DA postsynaptic receptors (Ackerman and White., 1992, Henry et al., 1989, Hopf et al., 2007) are directly related with behavioral sensitization induced by psychostimulants (Marin et al., 2008, Pierce and Kalivas, 1997). At the level of origin of dopamine system i.e. in the mid brain as well as in the terminal region serotonin has inhibitory influence on the activity of dopamine neurotransmission (Haleem, 2006). Therefore, activity enhancing effect of drugs of abuse could be modulated by serotonergic system (Hall et al., 2004, Przegaliski et al., 2000, Muller et al., 2003). The 5-HT2C receptor is one of the serotonergic receptor and this receptor activation by serotonin inhibits release of dopamine in different brain areas (Alex., 2005).

5-HT1A receptors role in the drug of abuse reinforcing effect was proposed because 5-HT1A receptors stimulation is capable of modulating cocaine-induced hyperactivity (De La Garza and Cunningham, 2000). CNS stimulants induced locomotor sensitization could be reduced by 5-HT1A receptor agonist administration. It has been reported that 5-HT1A receptor agonist osemozotan administration to mice sensitized by amphetamine inhibited long-term behavioral sensitization (Ago et al., 2008). Co-administration of 8-OH-DPAT a 5-HT1A selective agonist to rats injected with amphetamine (2.5mg/kg) cause reduction in the sensitization to amphetamine challenge dose (2.5mg/kg) (Przegaliski et al., 2000). Acute 8-OH-DPAT administration causes certain changes in cocaine induced patterns of locomotor activity (De La Garza and Cunningham, 2000).

Buspirone is partial agonist at 5-HT1A receptors and antagonist at dopamine D2 receptors (Gobert et al., 1999, Peroutka, 1985). Turnover of 5-HT is reduced when the animals were injected with buspirone suggesting that the buspirone could preferentially stimulate 5-HT1A somatodendritic receptors. Repeated buspirone administration decreased 5-HT1A somatodendritic receptor responsiveness (Haleem et al., 2007, Haleem and Khan., 2003, Bloise et al., 2007, Khan and Haleem, 2006). 5-HT1A somatodendritic receptors desensitization by buspirone co-administration will increase release of 5-HT at 5-HT2C receptors. 5-HT would be available resulting in an increase serotonin inhibitory influence on the dopaminergic neurons activity to reduce locomotor sensitization expression to psycho stimulants (Khan and Haleem, 2006).

Results from the present study on reduction of sensitization induced by methylphenidate could be explained as reversal of super sensitivity of soma to dendritic receptors. Buspirone is 5-HT1A somatodendritic receptors partial agonist with D2 receptors affinity, so it would be interesting to determine the role of post synaptic 5-HT1A and/or soma to dendritic receptors in the reduction of methylphenidate-induced sensitization by 8-OH-DPAT which is a full 5-HT1A agonist (Naidu and Kulkarni, 2001).

**CONCLUSION**

It supports the hypothesis that serotonergic inhibitory influence enhancement on the dopaminergic neurons activity may be the mechanisms due to which methylphenidate-induced motor sensitization could be attenuated by 5-HT1A receptor agonists. As methylphenidate repeated administration increases 5-HT1A somatodendritic receptors responsiveness and buspirone repeated co-administration decreases it, suggests that an increase in 5-HT1A somatodendritic receptors sensitivity play a crucial role in sensitization induced by methylphenidate. The findings may play an important role in the use of methylphenidate for the treatment of ADHD.

**REFERENCES**


