Attenuation of methylphenidate-induced tolerance on cognition by buspirone co-administration

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Abstract: Methylphenidate as a psycho stimulant drug has been prescribed in neuropsychiatric disorders to increase cognition and attention therefore is a medication of choice for attention-deficit/hyperactivity disorder however long-term administration of central nervous system stimulant produces tolerance on cognitive behavior. Previously it has been shown that long-term psychostimulant administration increases somatodendritic5HT-$_{1A}$receptors effectiveness. Repeated buspirone administration attenuates 5-HT$_{1A}$ soma to dendritic receptors effectiveness. This study was designed to determine that buspirone co-administration may reduce methylphenidate-induced tolerance on cognitive behavior. Cognitive effects were compared by using water maze and passive avoidance test weekly after long-term administration of methylphenidate, buspirone and their co-administration. Methylphenidate at a dose of 2.0mg/kg/day in rats initially improve memory but after long-term treatment produce tolerance on cognitive behavior this effect is more pronounce in case of spatial working memory of water maze test than passive avoidance learning memory. However oral buspirone co-administration at a dose of 10mg/kg/day prevents methylphenidate-induce tolerance on cognition. It is suggested that buspirone may oppose methylphenidate-induced cognitive tolerance by reducing the sensitivity of 5-HT1A soma to dendritic receptors. These findings may help to extend future therapeutics in ADHD.

Keywords: Methylphenidate, buspirone, cognition, passive avoidance test, water maze test, 5HT1A receptors.

INTRODUCTION

CNS stimulants are the drugs that increase intellect, cognition, performance and delay onset of fatigue. Despite these beneficial effects most stimulants are of little therapeutic value because long-term use of these drugs produces tolerance on cognitive behavior (Robinson and Berridge., 2000).

Methylphenidateis a medication of choice for persons suffering from (ADHD) attention-deficit/hyperactivity disorder (Sharma and Couture., 2014). Dopamine (DA) via its regulation of the prefrontal cortex through D1 and D2 receptors modulates cognitive performance (Goldman-Rakic., 1998). Methylphenidate increases DA signaling in the brain by blocking DA transporter (Barrett *et al*., 2005) thus enhances its extra cellular concentrations (Berridge *et al*., 2006). Methylphenidate is not only used to enhance attention and cognition in ADHD and other neuropsychiatric disorders (Clatworthy *et al*., 2009) but also abused to enhance cognitive functions by healthy individuals (Volkow and Swanson, 2008), yet there is increasing evidence that they do not promote learning (Advokat *et al*., 2008), which can be due to tolerance to the cognitive effect of the drug.

Central serotonergic system plays a critical role in memory processes regulation and in memory improvement from impaired cognitive performance (Mowla *et al*., 2007). Buspirone is 5-HT$_{1A}$ auto receptors agonist and an antagonist at certain postsynaptic 5HT$_{1A}$ receptor site (Zifa and Fillion, 1992) and rather than the postsynaptic D2 receptors it preferentially blocks presynaptic D2 receptors (McMillen and McDonald 1983). 5-HT$_{1A}$ somatodendritic inhibitory receptors that control release of 5-HT (Barnes and Sharp., 1999) are readily desensitized by chronic stimulation with 5-HT$_{1A}$agonist leads to increased release of 5-HT which results in activation of 5-HT$_{1A}$ postsynaptic receptors (Haddjeri *et al*., 1998).

Previously it has been shown that long-term psycho stimulant administration increases the effectiveness of somatodendritic5-HT$_{1A}$ receptors (Wdzony *et al*., 1997). Repeated buspirone administration reduces the effectiveness of 5-HT$_{1A}$ somatodendritic receptors. This study was designed to determine whether buspirone co-administration may attenuate methylphenidate-induced tolerance on cognitive behavior.

MATERIALS AND METHOD

Animals

Albino Wister rats bred locally, weighing 180-200g were individually housed under 12h dark and light cycles i.e. light on at 06:00h and room temperature controlled at 24+2^{o}C with free access to tap water and rodent diet cubes at least 7 days before starting the experiment to familiar them with the environment. They were accustomed to various handling procedures to nullify stress effects. All the performed experiments were according to the approved protocols and were in

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accordance to the rules and regulation given by local animal care committee

Behavioral assessment

Water maze test

Apparatus.

In the present study the Water Maze (WM) apparatus used consisted of a (60x30cms) transparent glass tank rectangular in shape filled with room temperature-water in addition to the powder milk, to the depth of 12cm. A wooden platform (15x13cms) was in a fixed location hidden 2cm below the surface of water.

Procedure

By assessing performance in a WM test the effects on spatial memory were examined. Initially and during the training session 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. Allowed the rat to stay for 10 seconds on the platform. Within the allowed time if the rat failed to find the platform it was guided gently onto the platform. By recording the retention latency, memory function of rats was tested. Retention latency is the time taken by each rat to locate the hidden platform 1 h (short term ST) 24h (long term LT) after training. 2 minutes was the cut off time for each session. Cognitive test of drug treated and control animals were monitored on 1st day and weekly during six weeks of treatment and short-term cognitive effect also monitored 1&1/2 hr. post haloperidol injection, at the same time to avoid time effect and to avoid order effect experiment is performed in a balanced design.

Passive avoidance test

Apparatus

Two compartments of passive avoidance (PA) paradigm include a dark 'punishable' and an illuminated ‘safe’ one. These two compartments were connected through a door, which enable free crossing of rats from one to another compartment. Grid floor is present in both compartments. The distance between the rods was 0.5cm with 5mm diameter of rods.

Procedure

Animals were introduced in an illuminated box during the session of training. Once the rats prompted into the dark compartment by their instinct stepped its four paws. Through the grid floor, animal received 1.5 mA foot shocks for 5 seconds to its paws. When receive the foot shock, immediately it came back to safe illuminated compartment. In the test period i.e. after 24 hour later, rats were again placed for a maximum of 5minutes in the bright compartment. Latency time before the rat entered the dark compartment indicate as the step- through latency which was recorded in the test session as described earlier (Khaliq *et al*., 2006). To avoid order effect passive avoidance test of all drug treated and control animals were performed weekly in a balance design.

Drugs

Methylphenidate was obtained from local medical store and prepared in 0.9% NaCl (saline) and buspirone (Research Biochemicals Incorporated) prepared in distil water. Drugs were administered by per oral route twice a day individually and also co-administered to the 3rd group of treated animals where as control animals were treated with saline (0.9%) per oral twice a day.

Experimental protocol

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 and saline to control rats orally two times daily (8.00 AM and 8.00 PM) for six weeks. Among the three groups of treated rats 1st group was given methylphenidate in the dose of 2mg/kg/day (0.18-0.2ml of methylphenidate suspension 2 times daily), 2nd group was given bupirone at the dose of 10mg/kg/day (0.9-1ml of buspirone suspension 2 times daily) and 3rd group was given methylphenidate at the dose of 2mg/kg/day (0.18-0.2ml of methylphenidate suspension 2 times daily), and bupirone at the dose of 10mg/kg/day (0.9-1ml of buspirone suspension 2 times daily) according to the weight of the rats. The tablets were powdered and 10mg methylphenidate tablet was added in 10ml 0.9% NaCl and 5mg buspirone tablet was added in 5ml distil water to make the suspension i.e. 1mg/ml, then calculated amount of suspension was administered to each rat with the feeding tubes. Control rats were given Saline 2.0 ml/kg/day i.e. 0.18-0.2ml 2 times daily according to the weight of the rats. Water Maze and Passive Avoidance test were monitored weekly. In a balanced design experiment was performed such that control and drug treated rats were monitored alternately to avoid the order effect.

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-bupirone on weekly cognitive activity in WM and PA test were statistically tested by three-way analysis of variance (ANOVA) repeated measure design to see the effects of various factors involved. Post hoc comparison by Newman-Keulstest was performed 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 short-term memory in water maze

Fig. 1 shows effects of repeated methylphenidate, buspirone and their co administration on cognition in WM

monitored on first day and weekly for 6 weeks. Data was analyzed by three-way ANOVA repeated measure design showed significant effects of methylphenidate (df=1,30, F=43, p<0.01), buspirone (df=1,30, F=55.7, p<0.01) and repeated monitoring (df=5,90, F=5.6, p<0.01). Interactions between buspirone* methylphenidate (df=1,30, F=57.3, p<0.01), week* buspirone* methylphenidate (df=5,30, F=5.5, p<0.01) and week* methylphenidate (df=5,30, F=6.34, p<0.01) were found to be significant and non-significant interaction between week*buspirone (df=5,30, F=2.4, p>0.05).

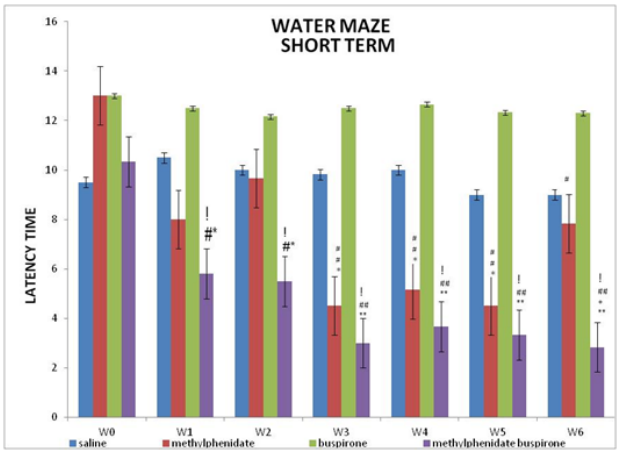

Fig. 1: Effect of methylphenidate, buspirone and their co-administration on short-term memory in water maze (from day 1 to 6th week). Values are means ± SD (n=8). Significant differences by Newman-Keuls test: +p<0.01 from similar week methylphenidate treated animals; !p<0.01from similar week buspirone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

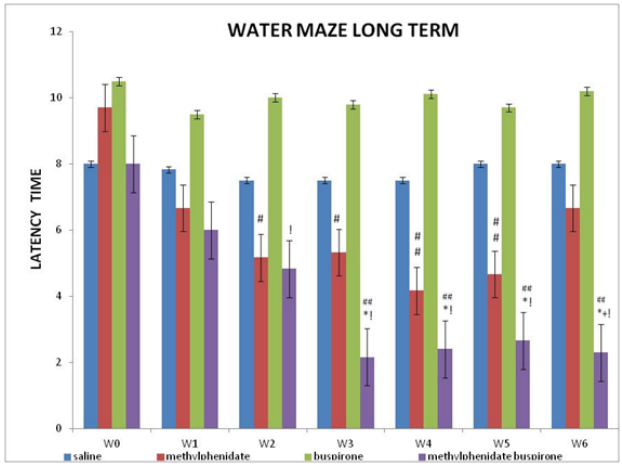

Fig. 2: Effect of methylphenidate, buspirone and their co-administration on long-term memory in water maze (from day 1 to 6th week). Values are means ± SD (n=8). Significant differences by Newman-Keuls test: *p<0.01 from similar week saline treated animals; #p<0.05, ##p<0.01 from similarly treated first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

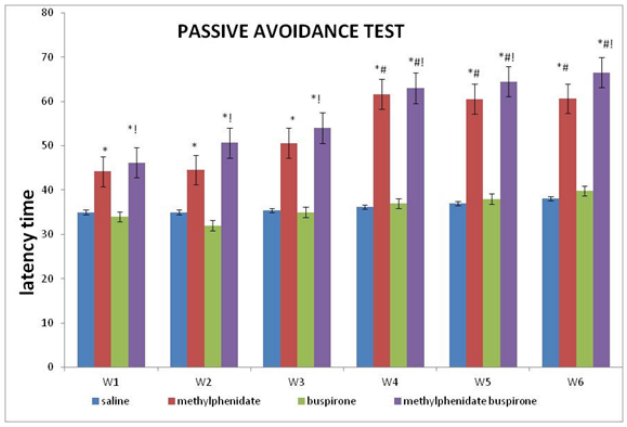

Fig. 3: Effect of methylphenidate, buspirone and their co-administration on performance of rats in passive avoidance test monitored weekly for 6 weeks. Values are means ± SD (n=8). Significant differences by Newman-Keuls test: *p<0.01 from similar week saline treated animals; #p<0.01 from similarly treated first day values;!p<0.01from similar week buspirone treated following three-way ANOVA (repeated measure design

Post hoc Newman-Keul test showed that methylphenidate administration significantly improve ST memory in 3rd, 4th and 5th week (P<0.05) from similar week control and (P<0.01) from similarly treated first day values and in 6th week from (P<0.05) first day values. Buspirone administration produce non-significant effect on memory in water maze but in co-administration buspirone enhanced cognitive effect of methylphenidate significantly (P<0.05) in week 1, 2 and significantly (P<0.01) in week 3, 4, 5 and 6 from similar week saline and from similarly treated first day values. By co-administration short-term memory improved significantly (P<0.01) in 1st, 2nd, 3rd, 4th, 5th and 6th week from similar week buspirone treated rats and in 6th week from similar week methylphenidate treated rats.

Effect of repeated administration of methylphenidate, buspirone and their co administration on long-term memory in water maze

Fig. 2 shows effects of repeated methylphenidate, buspirone and their co administration on cognition in WM monitored 24 hrs. after starting experiment and weekly for 6 weeks. Data was analyzed by three-way ANOVA repeated measure design showed significant methylphenidate (df=1,30, F=34.7, p<0.01) effect, buspirone (df=1,30, F=76.44, p<0.01) and repeated monitoring (df=5,90, F=24.03, p<0.01). Buspirone* methylphenidate interaction (df=1,30, F=31.96, p<0.01), week* buspirone (df=5,30, F=7.2, p<0.01), week* buspirone* methylphenidate (df=5,30, F=8.96, p<0.01)

and week* methylphenidate (df=5,30, F=14.99, p<0.01) were significant.

Newman-Keul Post-hoc comparison showed that methylphenidate administration improved LT memory significantly (P<0.01) in 4th and 5th week and significantly (P<0.05) in 2nd and 3rd week from their first day values. Buspirone administration produce non-significant effect on LT memory in WM but in co-administration buspirone significantly (P<0.01) enhance cognitive effect of methylphenidate in 3rd, 4th, 5th and 6th week from similar week saline and from similarly treated first day values. By co-administration LT memory improved. Significantly (P<0.01) in 2nd, 3rd, 4th, 5th and 6th week from similar week buspirone treated rats and significantly (P<0.05) in 6th week from similar week methylphenidate treated rats.

Effect of repeated administration of methylphenidate, buspirone and their co-administration on performance of rats in passive avoidance test

Fig. 3 shows effects of repeated methylphenidate, buspirone and their co administration on performance of rats in PA test monitored weekly for 6 weeks. Data was analyzed by three-way ANOVA repeated measure design showed significant methylphenidate (df=1, 30, F=72.71, p<0.01) effect, buspirone (df=1, 30, F=29.896, p<0.01) and repeated monitoring (df=5, 90, F=64.34, p<0.01). Buspirone* methylphenidate interaction (df=1, 30, F=163.41, p<0.01), week*buspirone (df=5, 30, F=9.00, p<0.01), week*buspirone*methylphenidate (df=5, 30, F=11.17, p<0.01) and week*methylphenidate (df=5, 30, F=12.46, p<0.01) were significant.

Newman-Keul post-hoc test showed that methylphenidate and co-administration of methylphenidate-buspirone significantly (P<0.01) improved memory from 1st week till 6th week as compare to controls and in 4th, 5th and 6th week from 1st week values. Memory improved significantly (P<0.01) in co-administration treated rats from 1st week till 6th week from similar week buspirone treated rats.

DISCUSSION

Methylphenidate has been shown to potentate the cognitive effect and is the main medication prescribed for ADHD (Sharma and Couture. 2014) to improve memory (Gonzalez-Garrido *et al*., 2009) attention and concentration (Bedard *et al*., 2007) yet there is increasing evidence that they do not promote learning (Advokat *et al*., 2008) which can be due to tolerance to the cognitive effect of the drug.

In the current study we chose WM and PA test to measure cognitive effect following methylphenidate, buspirone and their co-administration in rats. Methylphenidate administration in rats initially improve memory but after long-term treatment produce tolerance on cognitive behavior this effect is more pronounce in case of spatial working memory of WM test than PA learning memory.

Central dopaminergic systems play a critical role in the regulation cognitive behavior. Cognitive symptoms have been associated with dopamine disregulation in numerous diseases including schizophrenia (Knable and Weinberger, 1997) depression (Jimerson, 1987) drug addiction (Wise, 1996) and Parkinson disease. Methylphenidate blocks the DA transporter (Barrett *et al*., 2005) thus increasing the extracellular concentrations of DA. The memory improvement effect of methylphenidate has been attributed to the amplification of DA release in the central nervous system (Goldman-Rakic, 1998) and is used in the treatment of neuropsychiatric disorders like ADHD and other to enhance attention and cognition (Clatworthy *et al*., 2009).

Long-term psychostimulant administration increases the effectiveness of 5-HT_{1A}somatodendritic receptors (Wdzony *et al*. 1997). Presynaptic 5-HT_{1A} receptors activation causes reduction in serotonergic neurons firing rate of and decrease 5-HT synthesis, turnover, and release (Barnes and Sharp., 1999). Therefore, presynaptic 5-HT_{1A} receptor activation plays a role in cognitive deficits (Matsuda *et al*., 1995). 5-HT_{1A} receptor antagonist and partial agonists is capable of abolishing the psychostimulant-induced memory impairment in rats (Loscher and Honack, 1993).

Buspirone has partial 5-HT_{1A} receptors affinity as agonist (Wise, 1996). 5-HT turnover decreases when buspirone was injected, suggesting that the drug could stimulate 5-HT_{1A}somatodendritic receptors. Repeated buspirone administration decreased the responsiveness of 5-HT_{1A} somatodendritic receptor (Hensler, 2003). 5-HT_{1A} somatodendritic receptors desensitization bybuspirone co-administration will increase 5-HT release (Haddjeri *et al*., 1998). Previously reported that increase in brain serotonin activity is related to improvement in cognitive performance, whereas decrease 5HT levels in brain have been shown to impair memory function (Lieben *et al*., 2004). Our finding of increased cognitive performance in rats following long-term methylphenidate-buspironeco-administration support the contention that buspirone may oppose methylphenidate-induced cognitive tolerance by decreasing the sensitivity of soma to dendritic 5-HT_{1A} receptors (Hensler, 2003) leads to increase brain 5HT levels which have a positive effect on memory process (Laercio *et al*., 2004).

CONCLUSION

Attenuation of methylphenidate-induced tolerance on cognition by buspirone co-administration can be due to increase serotonin level or due to the reversal of sensitization of soma to dendritic 5-HT_{1A} receptors. The findings may have important consequences in the use of

methylphenidate as cognitive enhancer neuropsychiatric disorders.

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