Dose related neurochemical and behavioral effects of α-methylphenidate dose in rats

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Abstract— Central Nervous System stimulants are known to increase dopamine (DA) transmission. DA is a key neurotransmitter in cognition and motor activities. In animals, administration of stimulant drugs leads to enhance psychomotor response and dopamine release. This study examines the relationship between brain DA and 5-hydroxytryptamine (5HT) levels which are responsible for motor activity and cognition in rats.The present study compared the motor activity by using familiar and novel environments and cognitive effects by water maze procedure after long-term administration of oral therapeutic doses i.e. 2 mg/kg/day, 5mg/kg/day and 8mg/kg/day of methylphenidate in rats. Brain levels of 5HT, 5HIAA, DA, DOPAC and HVA analyzed by HPLC-EC. We found that with use of aforementioned doses increased brain DA and 5-HT levels. Dihydroxyphenylacetic acid (DOPAC) concentration decreased, and the concentration of homovanillic acid (HVA) increased dose dependently but no change was observed in 5-hydroxyindoleacetic acid (5-HIAA) concentration. Motor activity increased at higher dose (i.e.8mg/kg/day) as compared to the lower dose (i.e. 2mg/kg/day) of methylphenidate. Sensitization effects more pronounced after 20 days of drug administration were greater at smaller than higher doses. Smaller doses of drug (2mg/kg/day and 5mg/kg/day), but not higher doses (8mg/kg/day) improved performance in water maze.The behavioral effects of methylphenidate are explained in terms of DA and 5HT interaction involved in the control of motor activity and cognition.

Index Terms— Cognitive behavior , Dopamine , home cage activity, methylphenidate, oral doses, open field activity, serotonin, water maze.

1 INTRODUCTION

Psychostimulants such as methylphenidate, stimulate the central and peripheral nervous system [1]. To elicit an increase in arousal level and enhance physical and mental performance, the abuse liability of psycho-stimulant is well established and represents a significant public health concern [2].Development of locomotor sensitization to psychostimulant drug is an important predictor of psycho-stimulant drug abuse in animal models [3].An extensive literature documents the critical importance of dopamine (DA).[3] and serotonin (5 HT)[2] in the behavioral pharmacology and addictive properties of psycho-stimulants.

Methylphenidate is a piperidine derivative, structurally related to amphetamine, it elicits a behavioral profile that is very comparable to that of amphetamine[4]. Methylphenidate is a mild central nervous system stimulant however, large doses produce symptoms of generalized central nervous system stimulation and convulsions[5],[6]. Methylphenidate, also known as Ritalin, is widely used in the treatment of Attention Deficit Hyperactivity Disorder[7].

Long term use of methylphenidate causes dose-dependent sensitization[8],[9],[10],[11],[12]. Methylphenidate binds to DA transporters, resulting in an increase in synaptic availability of DA [13],[14]. Methylphenidate affects neurotransmission in brain regions including the prefrontal cortex (PFC) [15]. The mechanisms of sensitization to methylphenidate are not clear.[16]. The central DA system plays a crucial role in the psycho-stimulant-induced increase in motor activity as well as addiction[17],[3],[18]. Two major dopaminergic pathways in the brain namely nigrostriatal pathway and mesolimbic pathway are known to be involved in the regulation of motor behavior and emotional control respectively[19],[20]. In particular, the DA transporter plays a primary role in the reinforcing and behavioral-stimulant effects of psycho-stimulants in animals and humans[2]. Methylphenidate, a DA transporter blocker known to facilitate these cognitive processes[21],[22].

Purpose of our study was to monitor the effects of oral therapeutic doses of methylphenidate, on motor activity and memory function in relation to dopamine metabolism in rats. It was thought that long term use of methylphenidate which possibly produces sensitization may lead to tolerance in the ability of the drug elicit enhancement of learning and memory. A dose-dependent effect may therefore help to extend the therapeutic use of the drug for better clinical response.

2 Material and Methods

2.1 Test systems used *(Animals)*

Locally bred Albino Wister rats (weighing 180-200g) were housed individually under 12 h light and dark cycles (light on at 06:00h) and controlled room temperature (24+2℃) with free access to tap water and cubes of standard rodent diet at least 7 days before the start of experiment so that they could become familiar to the environment. They were accustomed to various handling procedures to nullify stress effects. All experiments were performed according to the protocols approved by the local animal care committee.

2.2. Materials for Behavioral assessment

2.2.1. Activity in a familiar environment (activity box)

Transparent Perspex cages (26x26x26 cm) with sawdust cover floor were used to monitor activity in familiar environment. Rats were placed individually in these cages to get familiar with the environment. After 15 minutes the numbers of cage-crossings were counted for 10 minutes[23].

2.2.2. Activity in the novel environment (open field)

A square area (76x76 cm) with walls 42 cm high was used to monitor activity in a novel environment. The floor of apparatus was divided by lines into 25 squares of equal sizes.
Animals were placed in the centre square of the open field. Latency to move and the numbers of square crossed with all four paws were counted for 5 minutes[24].

2. 2.3. Water Maze Test

The effects on spatial memory were examined by assessing performance in a Water Maze (WM) test. The Water Maze apparatus used in the present study consisted of a transparent rectangular glass tank (60x30 cms) filled with room temperature-water in addition to the powder milk, to the depth of 12cm.A wooden platform (15x13 cms) was hidden 2cm below the surface of water in a fixed location. Initially the rats were trained and during the training session each rat was placed into the water facing the wall of the tank and allowed 120 seconds to locate and climb onto the submerged platform. The rat was allowed to stay on the platform for 10 seconds. If it failed to find the platform within the allowed time it was guided gently onto the platform. Memory function of rats was tested by recording the retention latency (RL; the time taken by each rat to locate the hidden platform 1 h (short term) 24h (long term) after training. The cut off time for each session was 2 minutes.

2.3. Drugs

Methylphenidate HCl was obtained from local medical store and prepared in 0.9% NaCl (saline). Drug was administered in a volume of 1 ml/kg of body weight by per oral route twice a day to the treated animals and control animals were treated with saline (0.9%) at the dose of 1 ml/kg per oral twice a day.

2.4. Experimental protocol

Twenty-four male Albino Wister rats (weighing 180-220g) were randomly assigned to four groups, one control and 3 test groups, each containing six animals. The experimental protocol was designed to administer methylphenidate orally two times daily for 4 weeks.
Four groups were: (i) Saline (1.0 ml/kg/ day), (ii) Methylphenidate (2mg/kg/day) (iii) Methylphenidate (5mg/kg/day) (iv) Methylphenidate (8mg/kg/day) treated groups.
Behavioral Activities of rats i.e. activity in familiar environment was monitored on day 1, 5, 10, 15, 20 and 25 respectively, whereas activity in novel environment was monitored weekly and effects on spatial memory in Water Maze was monitored in last two weeks. . Rats were decapitated after 4 weeks to collect brain samples.
The experiment was performed in a balanced design in such a way that control and drug-treated rats were killed alternately to avoid the order effect. Brain samples were excised very quickly from the cranial cavity with in 30 seconds of the decapitation. Fresh brains were dipped in the chilled saline (0.9% w/v) and stored at low temperature (-70 ° c) until analysis of 5HT, 5HIAA, DA, DOPAC and HVA by HPLC-EC were carried out.

2.5. Statistical analysis

Results are represented as mean ±S.D. Statistical analysis of home cage, openfeild and water maze were performed by two-way analysis of variance (ANOVA) repeated measure, where as statistical analysis of brain DA, DOPAC, HVA, 5HT, and 5HIAA levels were performed by one-way analysis of variance (ANOVA) to see the effects of various factors involved. Post hoc comparison of groups was performed by Newman-Keul test. Values of $p<0.05$ and $p<0.01$ were considered as significant.

3. Results

3.1. Dose-related effect of methylphenidate administration on motor activity in familiar environment.

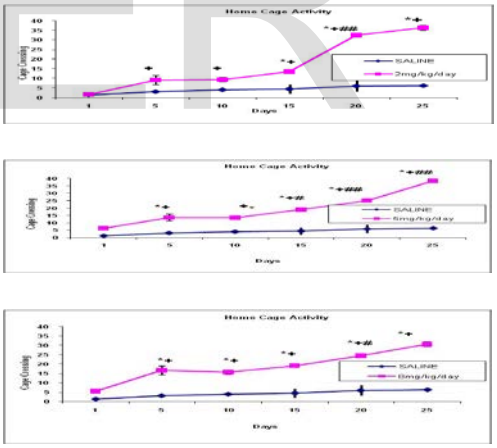


Fig. 1 A, B and C. Dose related (low dose(2mg/kg/day), moderate dose(5mg/kg/day), high dose(8mg/kg/day) effects of methylphenidate administration on motor activity in a familiar environment of skinner's box. Values are means ± SD (n=6).Significant differences by Newman-Keuls test: *P<0.01 from similar day saline treated rats.+ P<0.01 from day 1 similarly treated values of similarly treated rats. # P<0.05 , ## P<0.01 from similarly treated preceding days values following 2 way ANOVA (repeated measure design)

(Figure 1-A,B and C) Shows dose-related effects of methylphenidate in rats, on motor activity in familiar environment. Data analyzed by two-way ANOVA repeated measure showed a significant dose (i.e. F=97.3, df =3, 16, P<0.01) daily treatment (F=156.4, df =5, 16, P<0.01) effect and a signifi-

cant interaction between the two factors (i.e. F=20.3, df =15, 54, P<0.01).

Post hoc analysis by Newman-Keul test showed significant (P<0.01) increase in activity on day 15, 20 and 25 by low dose (2 mg/kg/day) whereas moderate (5mg/kg/day) as well as high (8mg/kg/day) dose of methylphenidate significantly (P<0.01) increased activities on day 5, 10, 15, 20 and 25 from similar day saline treated rats.

All three doses of methylphenidate (i.e. 2, 5, and 8 mg/kg/day) significantly (p<0.01) increase home-cage activity on days 5, 10, 15, 20 and 25 from first day values. Mean values of activity increases significantly (P<0.01) on day 20 by low dose (2mg/kg/day), where as moderate dose (5mg/kg/day) increase activity significantly (P<0.05) on day 15 and (P<0.01) and on day20 and 25, and high dose (8mg/kg/day) significantly (P<0.05) and (P<0.01) increased activity on day 20 and day 25 respectively from preceding week values.

3.2. Dose related effect of methylphenidate administration on motor activity in novel environment.

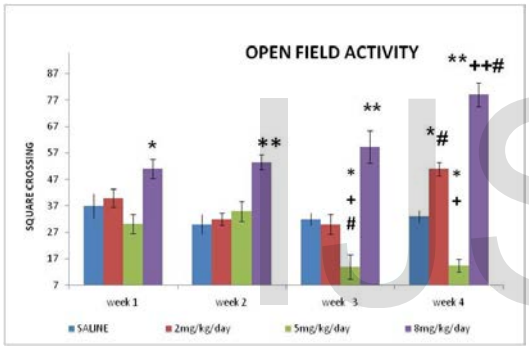


Fig.2 .Dose related effects of methylphenidate administration on activity in the novel environment of an open feild. Values are means ± SD (n=6).Significant differences by Newman-Keuls test: *P<0.05 **P<0.01 from similar week saline treated rats. +P<0.05, ++P<0.01 from week 1 values of similarly treated rats. . # P<0.01 from similarly treated preceding week values following 2 way ANOVA (repeated measure design)

Fig. 2 shows dose related effects of methylphenidate in rats, on motor activity in novel environment of an open field. Data of square crossing in open field (monitored weekly) analyzed by two-way ANOVA repeated measure showed a significant dose (F=20.14, df=3,18, P<0.01) and week (F=7.14, df=3,18, P<0.01) effect and also a significant interaction between the two factors (F=14.02, df=9,60, P<0.01).

Post hoc analysis by Newman-Keul test showed significant (P<0.05) increased in the square crossing by high (8 mg/kg/day) dose of methylphenidate in 1st week and by low dose (2 mg/kg/day) in 4th week and significantly (P<0.01) increased by high (8 mg/kg/day) dose in 2nd 3rd and 4th week, where as square crossing decreased significantly (P<0.05) in 3rd and 4th week by moderate dose (5 mg/kg/day) compared to similar week saline treated rats. Moderate dose (5 mg/kg/day) significantly (P<0.05) decreased square crossing in 3rd and 4th week where as high (8mg/kg/day) dose of methylphenidate increased significantly (P<0.01) in 4th week from first week values. On 3rd week there is significant (p<0.01) decrease in the square crossing by moderate dose (5 mg/kg/day) and in week 4th significant (p<0.01) increase in the square crossing by low dose (2mg/kg/day) and high (8mg/kg/day) dose of methylphenidate from preceding week values.

3.3. Dose related effect of methylphenidate administration on short term and long term memory in rats.

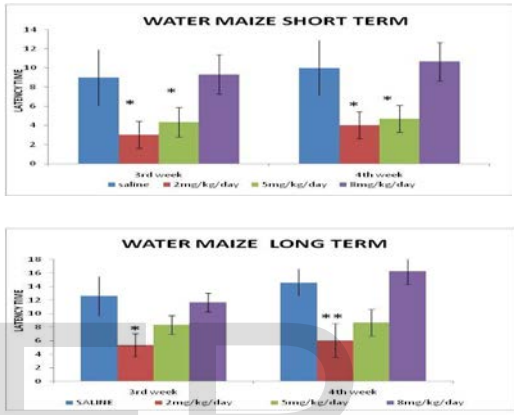
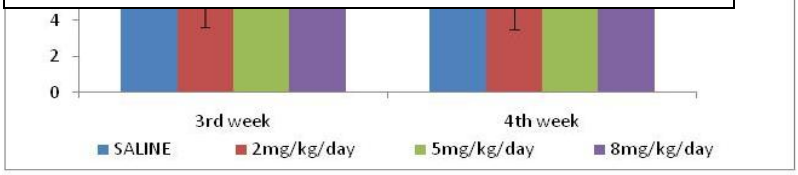


Fig.3.A and B. Dose related effects of methylphenidate administration on short term and long term memory on water maze in rats. Values are means ± SD (n=6). Significant differences by Newman-Keuls test: *p<0.05,**p<0.01 from similar week saline treated rats following 2 way ANOVA (repeated measure design)



Effects of methylphenidate-monitored 24 hours after training on water maze (see figure 3- B) analysis by two-way ANOVA repeated measure showed a significant dose effect (F=8.522, df=3,20, P<0.01) week (F=, df=1,20, P>0.05) effect and a significant interaction between the two factors (F=7.760, df=3,20, P<0.01).

Post hoc analysis by Newman-Keul test showed that long term memory was improved significantly (P<0.05) and (P<0.01)

following low dose (2mg/kg/day) in 3rd and 4th week respectively but not by moderate (5mg/kg/day) and high doses (8 mg/kg/day) of methylphenidate.

3.4. Dose related effect of methylphenidate administration on DA, DOPAC, HVA, 5HT, and 5HIAA levels in brain.

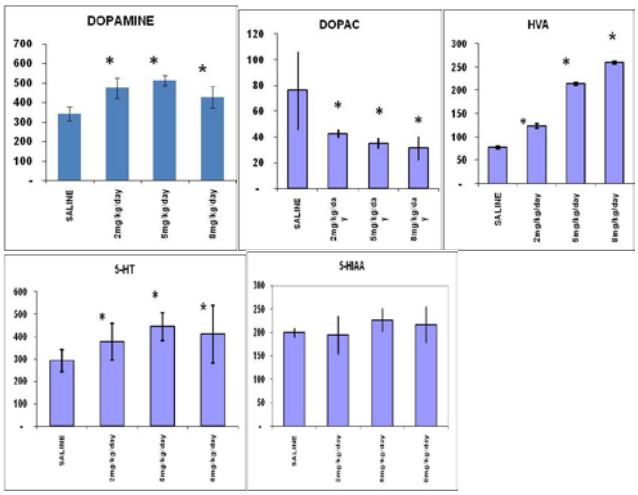


Fig.4 A, B, C, D & E. Dose related effects of methylphenidate on DA, DOPAC, HVA 5-HT & 5-HIAA levels in whole brain. Values are means ± SD (n=6). Significant differences by Newman-Keuls test:*P<0.01 from saline treated rats following 1 way ANOVA.

Data on brain DA, DOPAC, HVA, 5HT, and 5HIAA analyzed by one-way ANOVA (df= 3, 20) revealed significant effect of drug on brain DA (F=6.92, P<0.01), DOPAC (F= 11.88; p<0.01), HVA (F= 627; P<0.01) and 5HT (F=4.30; P<0.05). Effect of treatment on brain 5HIAA (F=1.63; P>0.05) was not significant.

Post hoc analysis by Newman-Keul test showed that brain concentration of DA, 5HT and HVA was significantly (P<0.01) increased and brain concentration of DOPAC was significantly decreased following low dose (2mg/kg/day), moderate dose (5mg/kg/day) and high dose (8mg/kg/day) of methylphenidate

4. DISCUSSION

Dose related effect of methylphenidate administration on motor activity

In the study we monitored the effect of Methylphenidate on the activity of rats in the familiar environment of home cage, and in the novel environment of open field, to compare the dose-dependent effect. Motor activity in home cage was monitored on every 5th day and activity in open field was monitored on day 1, 7, 14, and 21 to maintain the novelty of environment as frequent monitoring in open field could result in familiarization. Methylphenidate at different doses (i.e. 2mg/kg/day, 5mg/kg/ day, 8mg/kg/day) gradually increased motor activity in familiar environment as has been reported previously and produced sensitization after 2 weeks[25]. Several studies have reported sensitization development to the locomotor effects of Psychostimulants[8],[9],[10],[11],[12],[26].

Maximum increase in activity from day 1 occurred at a dose of 8mg/kg/day (high dose) in both familiar as well as in novel environment.Moderate(5mg/kg/day) and low (2mg/kg/day) doses also gradually and significantly increased activity in familiar environment, however low and moderate dose did not significantly increase activity in novel environment (see figure 2). The result may be explained in terms of anxiogenic effect of the drug reducing stimulatory effect in the novel environment. It has been suggested that drugs with brain/ behavioral effects similar to methylphenidate, increase fear and anxiety in rats [27],[28].

Although administration of drug at high dose (8mg/kg/day) did not decrease number of square crossed in novel environment. It is because the intense psychomotor stimulation hides anxiogenic effect of the drug. Indeed other authors also have reported increase anxiety and fear using various paradigms such as introduction of novel objects following methylphenidate administration [29],[30] and in alcove derive measures of behavior[31].

Dose related effect of methylphenidate administration on short term and long term memory in rats.

Methylphenidate has been shown to potentiate the cognitive effect and is the main medication prescribed for attention deficit hyperactivity disorder[32] to improve memory[33] attention and concentration [21], [34],[35], yet there is increasing evidence that they do not promote learning [35].

In the current study we chose water maze test to measure effects in memory function following methylphenidate administration at three different doses. In the Water Maze (WM) test the motivating stimulus is a hidden fixed platform. This task requires subject to use the spatial arrangement of cues outside of the rectangular pool to swim to the hidden platform. It was seen that methylphenidate decreased the latency time to reach the platform as compared to control rats, suggesting memory-enhancing effect of methylphenidate on WM assessed 24hrs after the trial. The rats treated with low dose (2mg/kg/day) took significantly lesser time in both weeks, whereas WM assessed I hr after the trial showed significant memory improvement in low dose (2mg/kg/day) as well as in moderate dose (5mg/kg/day) but not in high dose (8mg/kg/day). Other studies have also reported that working memory enhancement did not occur at high doses of methylphenidate[35],[36]. This may occur because hyperactivity may decrease attention for a particular task to mask cognitive effects of the drug.

Dose related effect of methylphenidate administration on DA, DOPAC, HVA, 5HT, and 5HIAA levels in brain.

Central serotonergic and dopaminergic system play a crucial role in the regulation of behavior[18]. We monitored DA, DOPAC, 5HT and 5HIAA levels in whole brain following daily administration of methylphenidate in three different doses for

4 weeks. We found an increase in brain DA and 5HT in all three doses. Other studies demonstrated that methylphenidate inhibits DA transporter and extracellular dopamine levels increases due to inhibition of its reuptake [13],[14]. Dose-dependent increased HVA levels show release of DA[37] and DA is less available to monoamineoxidase enzyme as reuptake decreased by the drug, there was dose dependent decrease in DOPAC levels (fig 4B) [38]. Increased DA levels by decreasing reuptake increases arousal levels that's why it is used in ADHD [39].

Previously it has been indicated that DA and 5HT system contribute separately to motoric activation, it is important to consider both DA and 5HT contributions to disorders of motoric impoverishment such as hyperkinetic states induced by stimulant drugs[20],[40],[41]. Serotonergic system is known to inhibit dopamine neurotransmission at the level of origin of dopamine system in the midbrain as well as in the terminal region[42].But in the present study whole brain was considered so the mechanism by which methylphenidate elicits hyperactivity may at least in part involve a decrease in the inhibitory influence of 5-HT on DA neurotransmission.

Results indicate that changes in DA and 5-HT metabolism are equal at all doses used in the study but enhancement of learning and memory exhibited only in low dose 2mg/kg/day not in high dose 8mg/kg/day[43],[44]. Several studies have reported memory deficit due to the increased DA and decreased DOPAC concentration in brain areas that are involved in cognitive process[45],[46]. It is suggested that high dose inhibit DA reuptake more strongly than low dose which is further confirmed by the dose dependent decrease in DOPAC levels however DA levels increases almost equally at all doses used in the study[47] (Tekes et al.,1988). Smaller inhibition of reuptake mechanism by low dose as compared to high dose is mainly responsible for smaller increase in activity which leads to significant improvement in memory whereas in high dose hyperactivity decreases cognition.

5. Conclusion

In summary this study provides the documentation of significant relationship between increase in brain DA and cognition and motor activity in response to different oral therapeutic doses of methylphenidate.Our results suggest a role of DA in the enhancement of cognition. Cognitive behavior and motor activity is not similar at all doses used in the study that reflect the difference inhibition of DA reuptake by the different doses of methylphenidate. These results also suggest that smaller dose i.e. 2mg/kg/day did not elicit sensitization and is probably devoid of abuse potential.

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