Effect of repeated oral therapeutic doses of methylphenidate on food intake and growth rate in rats

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Abstract: Central nervous system stimulants are known to produce anorexia. Previous data suggest that methylphenidate can have variable effects on caloric intake and growth rate. A dose-response study was performed to monitor caloric intake, liquid intake and growth rate in rats following repeated administration of human oral therapeutic doses 2 mg/kg/day, 5mg/kg/day and 8mg/kg/day of methylphenidate. We found that food intake and water intake, increased in all weeks and at all doses used in the study. Growth rate increased more at higher dose (8mg/kg/day) and at low dose (2mg/kg/day) of methylphenidate in 1st and 2nd week whereas more decreased by the above doses in 3rd week, suggesting that food stimulation leads to initial increase in growth rate but long term administration of methylphenidate attenuate growth rate that is not due to modulation of appetite but may be due to anxiety and increased activity produce by stimulants. A possible role of DA, 5HT receptors in modulation of appetite and anxiety is discussed.

Keywords: Methylphenidate, food intake, growth rate, water intake, dopamine.

INTRODUCTION

Methylphenidate HCl (MPH) is a psychomotor stimulant, which readily enters the brain to alter dopamine (DA) neurotransmission, used for the treatment of attention deficit hyperactivity disorder (Leddy *et al*., 2009; Volkow *et al*., 2012; Olfson *et al*., 2007). MPH binds to DA transporters, therefore DA neurotransmitter remain in the synaptic cleft for longer time, produces an indirect DA agonist effect (Howell *et al*., 2008).

Main concern exists about the use of MPH, in case of attention defect hyperactivity disorder (ADHD) or in case of hyperactive children, because of possible effects of MPH on general physical and emotional growth. There are evidences of retardation in height and weight increase (Aronsona, 2006). However, there is variance in the reported long-term ability of MPH to sustain weight loss ranging from 3months to the duration of administration of a clinically effective dose (Leddy *et al*., 2009; Barkley *et al*., 1990).

Stimulants are known to produce anorexia (Goldfield *et al*., 2011; Davis *et al*., 2012) but MPH can have variable effects on appetite (Işeri, 2007). Different factors can regulate intake of food including requirements of calories and reinforcing responses to food (Mietus-Snyder, 2008). One of the important neurotransmitters involved with feeding behaviors is dopamine, and its pharmacological manipulation has marked effects on food intake. MPH by blocking dopamine transporters enhanced dopamine signals in dorsal striatum and increase dopamine signals in dorsal striatum results in hyperphagia (Volkow *et al*., 2002). MPH induced an increase in hunger and desire for food can also explained in terms of a decrease in the availability of 5HT at the hypophagic serotonin receptors. The present study evaluate the effects of different oral therapeutic doses (human) on growth rate and intake of food and water following repeated administration of MPH in rats. In the view of the role of DA and 5HT on food intake we hypothesized that long-term administration of methylphenidate attenuates growth rate that is not due to modulation of appetite, may be due to anxiety and increased metabolism produced by the stimulant.

MATERIALS AND METHODS

Animals

Wister rats (weighing 180-200g, N=24) were used, locally bred and housed individually under light and dark 12 hour cycles (light on at 06:00h) and room temperature was controlled at (24+2^{o}C). They had free access to rodent diet cubes and tap water and 7 days prior to the start of the experiment they were familiarized to the environment. They were accustomed to various handling procedures to nullify stress effects. All experiments were performed according to the approved protocols of local animal care committee.

Measurement of growth rate

Rats were weighted before starting the experiment. Gain in body weight was monitored weekly during the four-week treatment. The growth rate of each rat was calculated in terms of change in body weight as percentage of preceding week's body weight.

Measurement of food intake

Amount of food intake was monitored weekly by giving rats weighted amount of food and weighting the remaining food in the hopper of the cage. The amount of

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food consumed was calculated by subtracting the amount of food left in the hopper from the amount of food placed in the hopper. Intake was calculated for whole week as % in preceding week mg/gm of body weight.

Measurement of water intake

Known volume of water was given in bottles. Intake of fluid was monitored weekly by measuring the water left in the bottle and calculates for whole week as % in preceding week mg/gm of body weight.

Drugs

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

Experimental protocol

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 administered methylphenidate orally two times daily for 4 weeks. Four groups are: (i) saline (1ml/kg/day) control group (ii) methylphenidate (2mg/kg/day) (iii) methylphenidate (5mg/kg/day) (iv) methylphenidate (8mg/kg/day) treated groups. Food intake, water intake and growth rate of rats were monitored weekly. After 4 weeks the rats were decapitated to collect brain samples. The experiment was performed in a balanced design in such a way that food intake, water intake and growth rate of control and drug treated rats were measured alternately to avoid the order effect.

STATISTICAL ANALYSIS

Results are represented as mean ±S.D. Analysis were performed by 2-way-ANOVA (2-way analysis of variance) to see the effects of various factors involved. Post hoc comparison was performed by Newman-Keuls test and $P<0.05$ and $P<0.01$ values were considered as significant.

RESULTS

Dose related effect of methylphenidate administration on growth rate.

Fig. 1 shows dose related effects of methylphenidate in rats, on growth rate. Data of growth rate in preceding weeks body weight analyzed by two-way ANOVA repeated measure showed a significant dose ($F=5.45$, $df=3,19$, $P<0.01$) week ($F=29.49$, $df=2,19$, $P<0.01$) effect and also a significant interaction between the two factors ($F=4.14$, $df=6,40$, $P<0.01$).

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

Dose related effect of methylphenidate administration on food intake % in preceding weeks in rats.

In fig. 2 shows dose related effects of methylphenidate on food intake % in preceding week's mg/gm of body weight. Analysis by two-way ANOVA repeated measure showed a significant dose ($F=32$, $df=3,19$, $P<0.01$), week ($F=782.8$, $df=2,19$, $P<0.01$) effect and also a significant interaction between the two factors ($F=10.79$, $df=6,40$, $P<0.01$).

Newman-Keul test post hoc analysis showed that food intake was significantly ($P<0.01$) improved following the daily administration of methylphenidate in low dose (2mg/kg/day) moderate dose (5mg/kg/day) and high dose (8 mg/kg/day) for one, two and three weeks compared to similar week saline treated rats and in 2nd and 3rd week from first week values. Food intake also increased significantly ($P<0.01$) in 3rd week by the three doses of methylphenidate from preceding week values.

Dose related effect of methylphenidate administration on water intake % in preceding weeks in rats.

In fig. 3 shows dose related effects of methylphenidate on water intake % in preceding week's mg/gm of body weight. Analysis by two-way ANOVA repeated measure showed a significant dose ($F=582$, $df=3,19$, $P<0.01$), week ($F=8416$, $df=2,19$, $P<0.01$) effect and also a significant interaction between the two factors ($F=389$, $df=6,40$, $P<0.01$).

Newman-Keul test post hoc analysis showed that water intake was significantly ($P<0.01$) improved following the daily administration of methylphenidate in low dose (2mg/kg/day) moderate dose (5mg/kg/day) and high dose (8 mg/kg/day) for one, two and three weeks compared to similar week saline treated rats and in 2nd and 3rd week from first week values. Water intake also increased significantly ($P<0.01$) in 3rd week by the three doses of methylphenidate from preceding week values.

DISCUSSION

Our current study examined the weekly effect on caloric intake, liquid intake and growth rate through daily oral

administration of methylphenidate. MPH at different doses (i.e. 2mg/kg/day, 5mg/kg/day, 8mg/kg/day) significantly increased food intake and water intake throughout the 3 weeks. Significant weight gain was observed following low dose (2mg/kg/day), and high dose (8mg/kg/day) during MPH treatment in 1st and 2nd week but significantly decreased by the above doses in 3rd week.

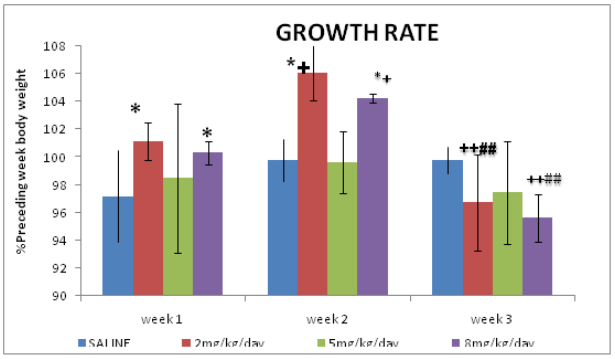

Fig. 1: Dose related effects of methylphenidate administration on growth rate. Values are means ± SD (n=6). Significant differences by Newman-Keuls test: *P<0.01 from similar week saline treated rats. +P<0.05, ++P<0.01 from week 1 similarly treated values. ## P<0.01 from similarly treated preceding weeks values.

The observed weight gain in 1st and 2nd week (fig. 1) is likely a result of increased in food intake, whereas long-term administration of MPH leads to weight loss. Other studies demonstrated that methylphenidate produces temporary retardation in height and weight gain (Aronsona, 2006). However, there is variance in the reported long-term ability of MPH to sustain weight loss ranging from three months to the duration of administration of a clinically effective dose (Leddy *et al*., 2009; Spencer *et al*., 1998; Barkley *et al*., 1990).

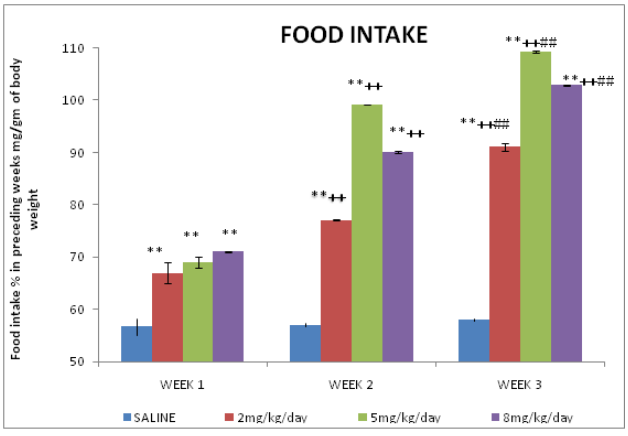

Fig. 2: Dose related effects of methylphenidate administration on Food intake. Values are means ± SD (n=6). Significant differences by Newman-Keuls test: **P<0.01 from similar week saline treated rats. ++P<0.01 from week 1 similarly treated values. ## P<0.01 from similarly treated preceding weeks values.

It has been shown that MPH which is a first line drug in the treatment of ADHD (Volkow *et al*., 2012;Olfson *et al*., 2007) leads to anxiety (Gadow *et al*., 2002; Vendruscolo *et al*., 2008) and significant increase in locomotor activity (Gerasimov *et al*., 2000). Additionally, as a psychomotor stimulant, MPH has been used clinically to treat narcolepsy (Roth, 2007). While locomotor activity and duration of sleep cycles were not quantified in this experiment, they are likely confounding factors responsible for the weight loss and should not be omitted from this discussion.

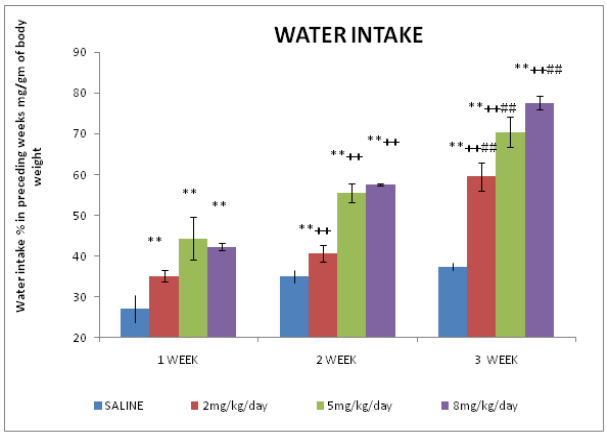

Fig. 3: Dose related effects of methylphenidate administration on water intake. Values are means ± SD (n=6). Significant differences by Newman-Keuls test: **P<0.01 from similar week saline treated rats. ++P<0.01 from week 1 similarly treated values. ## P<0.01 from similarly treated preceding weeks values

Stimulants are known to produce anorexia (Goldfield *et al*., 2011; Davis *et al*., 2012; Dourish, 1995; Sugrue, 1987). The important observation of this study is that the side effect of stimulants such as hyperphagia is not observed in all three therapeutic doses (fig. 2). Studies have shown that methylphenidate can have variable effects on appetite (Işeri, 2007; Wang *et al*., 2011). The drug which increases serotonergic effects produces anorexia (Blundell, 1991; Sugrue, 1987; Dourish, 1995). 5HT-1Band 5HT-2C are the main receptors that mediate the inhibitory response of 5HT on food intake (Clifton *et al*., 2003; Gibson *et al*., 1993; Kennett and Curzon, 1988). MPH induced hyperphagia, which can be explained in terms of a decrease in the availability of 5HT at the hypophagic serotonin receptors.

The mechanism by which MPH induces hyperphagia is not completely known, although it is attributed to being an effect of MPH action not only on serotonergic system but also on the dopaminergic system. MPH increases DA signaling through actions at the synapse, specifically,

through blockade of DA reuptake into the presynaptic terminal (Howell *et al*., 2008), increasing availability of pre-synaptic DA (Wilens, 2008). In the dorsal striatum dopamine is involved in food motivation in humans which is different from its role in regulating reward through the NA (Volkow *et al*., 2002). It is possible that MPH amplifies weak dopamine signals in dorsal striatum and therefore enhances the normal drive to eat (Volkow *et al*., 2002).

Studies have shown that MPH significantly increased water consumption (Rajala*et al*., 2012). Due to increased motor activity and also due to the reported adverse effect of the drug according to which MPH can produce dry mouth (Aronsona, 2006). This could have resulted from a change in the animals' ability to remain on task or from changes in the processing of rewards/failures (Rajala*et al*., 2012).

In conclusion, the results of this exploratory study indicate that long term administration of MPH significantly reduce growth rate that is not due to modulation of food intake but may be due to anxiety or increased locomotor activity. A better understanding of these mechanisms could illuminate new targets for future drug therapies for the treatment of ADHD.

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