Methylphenidate increases the urinary excretion of vanillylmandelic acid in rats that is attenuated by buspirone co-administration

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Abstract: Methylphenidate is a psychostimulant used for the treatment of (ADHD) attention deficit hyperactivity syndrome in children and adults. After chronic administration it is known to produce behavioral disorders including anxiety. Previous studies demonstrated that co-administration of buspirone can reduce behavioral and cognitive adverse effects produced by methylphenidate. The aim of the present study is to measure the levels vanillylmandelic acid (VMA) excretion in urine following prolong administration of methylphenidate, buspirone and their combination. Samples of urine for the estimation of the urinary VMA excretion were collected from treated and control male Wistar rats. We found significant ($P<0.01$) raised urinary VMA excretion in methylphenidate group however significant ($P<0.01$) reduction in VMA levels were seen after buspirone co-administration. Excretion of VMA in urine would allow the monitoring of sympatho-adrenomedullary system activity. This study could be helpful to increase the clinical use of methylphenidate in the treatment of different disoders.

Keywords: Oral administration, methylphenidate, vanillylmandelic acid, urinary excretion, buspirone.

INTRODUCTION

Central nervous system stimulants can attenuate the onset of fatigue, increase performance and intellect, despite all these useful effects stimulants are consider to be of less clinical importance as their prolong administration results in different behavioral problems including anxiety (Motaghinejad *et al*., 2015). Stress or anxiety result in sympatho-adrenomedullary system activation and after disturbance within seconds catecholamine are secreted and urinary metabolite exertion increased (Moberg 2000). Measurement of urinary (VMA) vanillylmandelic acid expose peripheral sympathetic nervous system activity, may indicate an important marker of anxiety,

Methylphenidate, an amphetamine like drug, administered for the ADHD treatment in children. Mechanism of methylphenidate is that it binds the dopamine, norepinephrine, and to lesser extent serotonin transporter and inhibit reuptake of these amine into synaptic terminals, thus stimulates their receptors. Chronic abuse of methylphenidate and its neurochemical and behavioral consequence in adult and children remain unclear (Challman and Lipsky, 2000). Buspirone is an anxiolytic drug that does not cause dependence. It is also effective in the treatment of depression in combination with other antidepressants (Albert and François, 2010; Albert and Fiori, 2014). Buspirone antagonizes presynaptic dopamine autoreceptors and act as a 5-HT_{1A} inhibitory autoreceptors agonist. Thus, buspirone respectively enhances and depresses the firing rates of both type of neurons (Davari-Ashtiani *et al*., 2010). Recent studies suggested that buspirone has both anxiolytic and antidepressant effect, thus it is one of the best choice to treat such situations where there are both anxiety and depressive symptoms like as amphetamine and other psychostimulant abuse (Butkevich *et al*., 2017).

Amphetamines like drugs in chronic use interfere with catecholamine and their metabolite results in an increased excretion of VMA in urine (Dobri 2014). Methylphenidate-induced pharmacological and behavioral responses are associated with elevated synthesis and utilization of catecholamine that leads to urinary excretion of hormone metabolites (Alvarenga *et al*., 2006). Catecholamines like noradrenaline, adrenaline and dopamine are inactivated by methylation forming metanephrines that can be sulphated or formerly metabolized by monoamine oxidases in vanillylmandelic acids and homovanillic acid (VMA and HVA) respectively (Corcuff *et al*., 2017). In reducing psycho-stimulants induced adverse effects buspirone co-administration produce an important role (Alam *et al*., 2015, Alam *et al*., 2016). Current study was designed to explore in what manner chronic administration of methylphenidate impacts the metabolism of

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catecholamine and to find out the role of buspirone co-administration on minimizing methylphenidate induced excessive release of catecholamine and VMA excretion.

MATERIALS AND METHODS

Animals

In the present experiment adult male Wistar rats of age 6 to 8 weeks were used. Selected animal's weighs ranged from 150 to 250 g. Animals were kept under standard environmental conditions, temperature: 21±1°C, humidity: 55±5% with 12:12 h light/dark cycle at the Department of Pharmacology, Faculty of Pharmacy, University of Karachi. The animals were maintained with free access to water and regular rat diet ad libitum. They were caged in pair and were acclimatize at least 7 days before the start of experiment. Animals were handled as per specification provided in Helsinki Resolution 1964. The experiment was performed according to the approved protocol and was in accordance to the rules and regulation given by the local animal care committee.

Drugs

Methylphenidate HCl prepared in saline NaCl 0.9% (Research Biochemicals Incorporated) and buspirone prepared in distil water (Research Biochemicals Incorporated) were used in the treatment.

Experimental protocol

In the present experiment animals divided into four groups as follows:

Group 1: saline treated (n=10)
Group 2: Methylphenidate treated (n=10)
Group 3: Buspirone treated (n=10)
Group 4: Methylphenidate + Buspirone treated (n=10)

Methylphenidate was given 2mg/kg/day (Aoyama *et al*., 1990; Wargin *et al*., 1983) buspirone was given 10mg/kg/day (Naito *et al*., 2003; Vis *et al*., 2001) and methylphenidate + buspirone at the dose of 2mg/kg/day, 10mg/kg/day respectively in combination. Control rats were given saline 2.0ml/kg/day. Calculated amount of suspension was given orally according to the weight of each Wister rat with the feeding tubes twice a day (8.00 AM and 8.00 PM) for 6 weeks.

Urine sample collection

The animals were received the respective treatment for 6 weeks. After 6 weeks all animals were placed into metabolic cages for urine collection for the assessment of urinary excretion of VMA. Pelleted food and bottled tap water were supplied ad libitum. Urine was collected from all animals at (at 6:00 a.m.) to avoid diurnal fluctuation from metabolic cages at ambient temperature and centrifuged at 13,000 rpm at 4°C for 5 min, and the supernatants were stored frozen at −20°C until VMA Analysis (Zhang *et al*., 2014).

STATISTICAL ANALYSIS

Results were compiled by using the SPSS software (version 16.0) and are expressed as mean ± S.D. The data on the effects of repeated methylphenidate, buspirone and their co administration on VMA was performed by one-way ANOVA analysis of variance. Post hoc was performed by the Newman-Keul test showed significance of $P<0.01$.

RESULTS

Effect of repeated administration of methylphenidate, buspirone and their co administration on urinary VMA levels

Data on the effects of repeated methylphenidate, buspirone and their co administration on levels of VMA in urine was analyzed by one-way ANOVA (df = 3, 12) revealed significant effects of drug treatment on the levels of urinary VMA ($F = 43.4$, $P<0.01$).

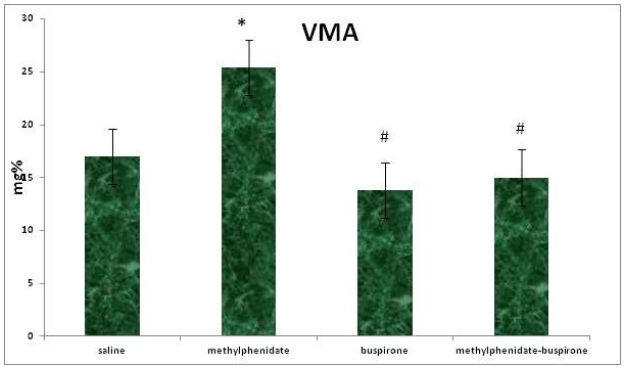

Fig. 1: Effect of methylphenidate, buspirone and co-administration of methylphenidate-buspirone on levels of VMA in urine in rats after 6 weeks of drug administration. Values are means ±SD (n=10). Significant differences by Newman–Keuls test: *P<0.01 from saline treated rats. #P<0.01 from methylphenidate treated rats following one-way ANOVA.

Post hoc performed by Newman-Keul test showed that the levels of VMA in urine significantly ($P<0.01$) increased in methylphenidate treated rats than saline treated rats and significantly ($P<0.01$) decreased in buspirone and methylphenidate-buspirone treated rats compare to methylphenidate treated rats.

DISCUSSION

Prolong use of psychostimulants are known to produce behavioral deficits. It has been shown previously that excretion of VMA, a catecholamine metabolite remained unchanged after acute administration of amphetamine (Chuang. 1981). However presently in our study urinary excretion of VMA has increased following long-term methylphenidate administration but co-administration of

buspirone significantly reduced the urinary VMA levels induced by methylphenidate.

In the central nervous system, catecholamines are important neurotransmitters that also play a critical role in the autonomic regulation of different homeostatic functions, namely, intestinal and bronchial smooth muscle tone, glucose metabolism, cardiac rate and contractility and vascular tone. Their effects are mediated via dopamine, alpha- and beta-adrenergic receptors (McDougall and McLeod., 1996). Catecholamines are present normally in little amounts in the plasma, but their levels can increase rapidly due to change in temperature, posture, emotional and physical stress (Hernandez *et al*., 2000). Catecholamines are metabolize to VMA in peripheral nervous system. Peripheral catecholamine metabolism results in excretion of VMA in urine (Hughes *et al*., 2004; Corcuff *et al*., 2017).

Stress or anxiety result in sympatho-adrenomedullary system activation and after disturbance within seconds catecholamines are secreted that results in excretion of elevated urinary metabolite (Moberg 2000). Measurement of urinary VMA, an important marker of anxiety exposes peripheral sympathetic nervous system activity. Since higher levels of urinary VMA excretion were associated with higher levels of anxiety (Hughes *et al*., 2004). This study demonstrated that buspirone can alter methylphenidate induced upregulation of catecholamine which we can be measured by assessing VMA level in urine.

Our results are in agreement with previous researches that explain psychostimulants can cause long-lasting neurochemical and behavioral adaptations by elevation in catecholamine levels (Martins *et al*., 2006). Methylphenidate inhibits dopamine and norepinephrine reuptake into presynaptic terminals as it binds the dopamine, and to a lesser extent the norepinephrine transporter protein (Sánchez-Pérez *et al*., 2012). Methylphenidate-induced pharmacological and behavioral responses are believed to be associated with increased catecholamine utilization and synthesis that results in the excretion of hormone metabolites in the urine (Zametkin *et al*., 1985)

In present study buspirone and methylphenidate co-administered group significantly reduced VMA concentration in urine which indicates that buspirone can revert undesirable effects of methylphenidate after chronic administration. Buspirone act as a partial agonist of postsynaptic serotonin $5HT_{1A}$ receptors and full agonist of presynaptic $5HT_{1A}$ receptors (Neppe, 1990). It has been shown previously that prolong administration of oral methylphenidate produces behavioral sensitization that was reduced by co-administration of buspirone so in reducing psycho-stimulants induced adverse effects, buspirone play an important role (Alam *et al*., 2015, Alam *et al*., 2016). Increase excretion of catecholamine metabolite in urine can be explained in terms of increase firing rates of central and peripheral catecholamine (Alvarenga *et al*., 2006). Decreased metabolite elimination in urine by co-administration of buspirone and methylphenidate indicate serotonergic inhibitory influence.

CONCLUSION

In conclusion, the ability of methylphenidate to increase VMA excretion may involve in mediating some of the biochemical and behavioral effects of methylphenidate which can be attenuated by buspirone co administration.

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