Methylphenidate Increases cGMP Levels in Rat Brain

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ABSTRACT:
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Objective: Methylphenidate (MPH) is commonly and effectively used to treat children and adolescents with attention-deficit hyperactivity disorder. However, MPH still poses a number of questions on which mechanisms of effect it has on the brain. The present study addresses the question of whether MPH induces cyclic guanosine 3',5'-monophosphate (cGMP) in rat brain. **Methods:** MPH at a dose of 10mg /kg p.o. was administered to rats daily for 8 weeks, whereas control rats were given distilled water. The level of cGMP was measured in total brains of the rats. **Results:** Brain cGMP levels were higher in the study group when compared with the control group (p=0.004). **Conclusions:** Our results suggested that the long term MPH administration might increase cGMP activity in rat brain.

Key words: methylphenidate, cGMP, secondary messenger

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INTRODUCTION

Methylphenidate [dl-threo-methyl-2-phenyl-2-(2-piperidyl) acetate] (MPH) is a mild central nervous system stimulant that has been widely used since the 1960s as the best available pharmacotherapy in the treatment of children and adolescents with attention-deficit hyperactivity disorder (1,2). However, yet little is known about mechanisms contributing to stimulant's therapeutic efficacy (3).

MPH promotes neurochemical effects, including dose-dependent increases in extracellular dopamine (DA) (4,5,6) and norepinephrine (NE) (3,4,5,7,8), both of which may be implicated in stimulant therapeutic actions (9). Nevertheless, the magnitude of the catecholamine responses with MPH for a behaviorally comparable dose was considerably less than that with amphetamine (4). In contrast, MPH is substantially less or no potent in inhibiting serotonin uptake compared with the catecholamines (4,10). Moreover, it has been proposed that an increase in histamine because of blocking the vesicular monoamine transporter type 2 by MPH (11). However, it's not completely unknown if other neurotransmitters also would be changed by MPH.

These neurotransmitters including DA, NE, serotonin, histamine induced by MPH are bound to the receptors that associated with G proteins (See Table 1). Activated G proteins (first messenger) in turn activate or inhibit effectors enzymes, such as adenylate cyclase, phospholipase C, and then produce soluble second messengers. Thus, second messengers for MPH are cyclic adenosine monophosphate (cAMP) via adenylate cyclase and inositol triphosphate / diacylglycerol (IP3/DG) via phospholipase C (12). As far as we know, in the current literature, there is no knowledge about whether MPH induces cyclic guanosine 3',5'-monophosphate (cGMP) as another seconder messenger, which is synthesized by guanylate cyclase.

The purpose of the current study

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was to determine sought to determine the activity of cGMP in rat brains after chronic MPH administration.

METHODS

Subjects

Subjects were 20 four-week-old Wistar male rats (120±15 g) housed individually in wire-topped cages, as five animals per cage. After seven-day acclimatization period, the rats were randomly assigned to two groups of 10 rats per group. Control and experimental rats received a standard diet of rodent chow (12-15 g/d) and water as they took. All rats were kept on an alternating 12-hour-light and 12-hour-dark cycle. The temperature inside the chambers was 22 °C (± 2) with relative humidity from 40 to 60%. The study group was administered 10 mg/kg/d of MPH, whereas the control group was administered distilled water.

All experiments were performed at the same time every day and in the light period (9.00-11.00 AM) during 8 weeks. The experiments have been carried out according to rules in the Guide for the Care and Use of Laboratory Animals adopted by National Institutes of Health (USA) and the Declaration of Helsinki. This study was approved by the Ethics Committee of Gulhane Military Medical Academy Research Center.

Drug Administration

Distilled water (0.5 ml) via orogastric intubation was given to each rat in the control group. MPH at a dose of 10 mg/kg was administered to those of the study group. The dosage of MPH administration to rats is similar to those of Gerasimov et al (6) and Brandon et al (13).

Ten mg tablets of MPH (Ritalin“) were dissolved in sterile distilled water via centrifugation providing 20 mg of MPH per 10 ml. 0.5 ml of the solution (contains MPH 1 mg) to each rat in the study group was administered for 8 weeks between 9.00 AM and 11.00 AM once a day, via orogastric intubation. The aim of intubation by using orogastric applicator was to prevent any drug loss due to uncontrollable reasons.

Tissue sampling

Three rats died (2 from the study group and 1 from the control group) because of the trauma during application of the orogastric apparatus and the study was completed with 17 rats.

At the end of 8 weeks, animals were decapitated and brains were removed. The brain tissues were frozen immediately after the sampling and kept at -70°C for chemical analysis. Tissue samples were weighed analytically. Then nine fold 1.15% KCl solution was added to the tissues and homogenized in glass homogenizer in ice. The homogenisated samples were centrifuged at +4°C at 4400xg for ten minutes. Then supernatant was used for the analysis.

Laboratory Methods

At the end of 8 weeks, cGMP levels in total brain of the rats were measured.

The cGMP levels were measured in total brain using an EIA kit (Assay Designs Inc., MI, USA). Measurements were done following the kit procedure as previously described (14).

Statistical Analysis

Results were expressed as mean and standard error (of the mean) (SEM). Differences between the two groups were analyzed using Mann-Whitney U test. A significance level of $p<0.05$ was considered to be statistically significant.

RESULTS

Brain cGMP levels were higher in the study group when compared with the control group (18.80±1.30 U/g and 11.42±1.31 U/g, respectively) (Z=-2.896, p=0.004) (Figure 1).

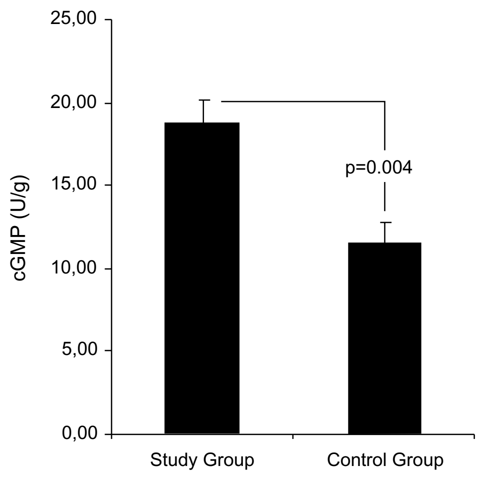

Figure 1. CT scan image

DISCUSSION

In our study, the significantly elevated cGMP activity in rat brains administered 10 mg/kg MPH for 8 weeks showed that this agent also might induce cGMP as another secondary messenger as well as cAMP and IP3. Table 1 shows neurotransmitters and its effector systems (first and second messengers) in brain (12). As shown in Table-1, acetylcholine and nitric oxide (NO) cause to increase cGMP activity. However, MPH does not affect acetylcholine (11). Thus, the increase in cGMP activity in our study is most likely to be related to an increase of nitric oxide (NO), which might be stimulated by MPH.

The free-radical gas, NO is synthesized from the precursor L-arginine by enzyme NO synthase. NO diffuses readily within cells and between cells, and among its activities is the potent activation of guanylate cyclase. NO causes an increase in the concentration of cGMP. The effects of NO are thought to be mediated by elevation of intracellular cGMP (15,16).

NO appears to be a messenger molecule in the central nervous system, fulfilling most of the criteria of a neurotransmitter (17). Several studies have shown that NO modulates the release of various neurotransmitters such as DA, NE, and glutamate, (18,19,20). Therefore, NO may be involved in the process that MPH increases dopamine DA and NE (5,7). Methamphetamine, which is a similar stimulant to MPH, was shown increases cerebellar levels of cGMP in mouse (21). Moreover, it has also been suggested that there is an interaction at the behavioral level between NO and the DA-mediated effects of amphetamine (22).

Tablo 1. Receptor Subtypes and Effector Systems for Neurotransmitters (12)

Neurotransmitter	Receptor Subtype	G/I[a]	Second Messenger[b]
Acetylcholine	M_1	G	IP_3/DG, increase cGMP
	M_2	G	Decrease cAMP, increase K^+ conductance
	M_3	G	IP_3/DG, increase cGMP
	M_4	G	Decrease cAMP
	M_5	G	IP_3/DG
	Nicotinic	I	Na^+/K^+
Dopamine	D_1,D_5	G	Increase cAMP
	D_2	G	Decrease cAMP, increase K^+ conductance
	D_3,D_4	G	? Decrease cAMP
Epinephrine and Norepinephrine	$\alpha1_{a, b, c, d}$	G	IP_3/DG
	$\alpha2_{a, b, c,}$	G	Decrease cAMP, increase K^+ conductance
	ß1, 2, 3	G	Increase cAMP
Histamine	H_1	G	IP_3/DG
	H_2	G	Increase cAMP
	H_3	?	?
Serotonin	5-HT_{1A}	G	Decrease cAMP, increase K^+ conductance
	5-$HT_{1B, 1E, 1F}$	G	Decrease cAMP
	5-$HT_{1C, 2A, 2B, 2C}$	G	IP_3/DG
	5-$HT_{1D, 4, 6, 7}$	G	Increase cAMP
	5-HT_3	I	Na+/K^+
	5-$HT_{5A, 5B}$	G	?
Nitric Oxide	-	-	increase cGMP
Glutamate	NMDA	G	Ca^{++}
	AMPA	G	Cation conductance
	Kainate	G	Cation conductance
	Metabotropic	G	IP_3

[a]G, G-protein-linked; I, direct linkage to an ion channel.
[b]IP_3, stimulation of phosphoinositide turnover, resulting in an increase in the concentrations of inositol triphosphate and diacylglycerol.

Our study had several limitations. First, we administered single MPH dose of 10 mg/kg once a day, second the number of the subjects were low, and third we could not examine nitrate-nitrite or NO levels if increased cGMP is result from NO. The possible effects of MPH on rat brain might be interpreted more accurately if different dosages were used; the number of the subject increased, and particularly nitrate-nitrite or NO levels were measured. Despite these limitations we found this study as an informative research for being the first study suggesting an effect of MPH on cGMP as a seconder messenger.

The results of the present study suggest that MPH administration at dose of 10 mg/kg in rats can alter cGMP levels/activities of brain. Further large-scale clinical and experimental studies are now needed to replicate our results.

References:

1. Challman TD, Lipsky JJ. Methylphenidate: its pharmacology and uses. Mayo Clin Proc 2000; 75:711-721

2. Biederman J, Mick E, Faraone SV. Age-dependent decline of symptoms of attention deficit hyperactivity disorder: impact of remission definition and symptom type. Am J Psychiatry 2000; 157:816-818

3. Kuczenski R, Segal DS. Exposure of adolescent rats to oral methylphenidate: preferential effects on extracellular norepinephrine and absence of sensitization and cross-sensitization to methamphetamine. J Neurosci 2002; 22:7264-7271

4. Kuczenski R, Segal DS. Effects of methylphenidate on extracellular dopamine, serotonin, and norepinephrine: comparison to amphetamine. J Neurochem 1997; 68:2032-2037

5. Kuczenski R, Segal DS. Locomotor effects of acute and repeated threshold doses of amphetamine and methylphenidate: relative roles of dopamine and norepinephrine. J Pharmacol Exp Ther 2001; 296:876-883

6. Gerasimov MR, Franceschi M, Volkow ND, Gifford A, Gatley SJ, Marsteller D, Molina PE, Dewey SL. Comparison between intraperitoneal and oral methylphenidate administration: a microdialysis and locomotor activity study. J Pharmacol Exp Ther 2000; 295:51-57

7. Gatley SJ, Pan D, Chen R, Chaturvedi G, Ding YS. Affinities of methylphenidate derivatives for dopamine, norepinephrine and serotonin transporters. Life Sci 1996; 58:231-239

8. Solanto MV. Neuropsychopharmacological mechanisms of stimulant drug action in attention-deficit hyperactivity disorder: a review and integration. Behav Brain Res 1998; 94:127-152

9. Biederman J, Spencer T. Attention-deficit/hyperactivity disorder (ADHD) as a noradrenergic disorder. Biol Psychiatry 1999; 46:1234-1242

10. Andersen PH. The dopamine inhibitor GBR 12909: selectivity and molecular mechanism of action. Eur J Pharmacol 1989; 166:493-504

11. Liu Y, Peter D, Merickel A, Krantz D, Finn JP, Edwards RH. A molecular analysis of vesicular amine transport. Behav Brain Res 1996; 73:51-58

12. Kaplan HI, Sadock BJ. Synopsis of Psychiatry. 8th Ed. Baltimore: Williams & Wilkins 1988:103-104

13. Brandon CL, Marinelli M, Baker L, White FJ. Enhanced reactivity and vulnerability to cocaine following methylphenidate treatment in adolescent rats. Neuropsychopharmacology 2001; 25:651-661

14. Aydın A, Orhan H, Sayal A, Özata M, Şahin G, Işımer A. Oxidative stress and nitric oxide related parameters in type II diabetes mellitus: effects of glycemic control. Clin Biochem 2001; 34:65-70

15. Dawson VL, Dawson TM. Physiological and toxicological actions of nitric oxide in the central nervous system. Adv Pharmacol 1995; 34:323-342

16. Denninger JW, Marletta MA. Guanylate cyclase and the NO/cGMP signaling pathway. Biochim Biophys Acta 1999; 1411:334-350

17. Synder SH, Bredt DS. Nitric oxide as a neuronal messenger. Trends Pharmacol Sci 1991; 12:125-128

18. Lonart G, Cassels KL, Johnson KM. Nitric oxide induces calcium-dependent [3H] dopamine release from striatal slices. J Neurosci Res 1993; 35:192-198

19. Montague PR, Gancayco CD, Winn MJ, Marchase RB, Freidlander MJ. Role of NO production in NMDA receptor-mediated neurotransmitter release in cerebral cortex. Science 1994; 263:973-977

20. Silva M, Rose S, Hindmarsh J, Aislaitner G, Gorrod J, Moore P, Jenner P, Marsden C. Increased striatal dopamine efflux in vivo following inhibition of cerebral nitric oxide synthase by the novel monosodium salt of 7-nitro indazole. Br J Pharmacol 1995; 114:257-258

21. Rao TS, Cler JA, Mick SJ, Iyengar S, Wood PL. Polyamines modulate events mediated by the N-methyl-D-aspartate (NMDA) receptor complex through an ifenprodil-insensitive pathway: in vivo measurements of cyclic GMP in the cerebellum. Neuropharmacology 1991; 30:567-573

22. Przegalinski E, Filip M. Nitric oxide (NO) pathway and locomotor hyperactivity towards dopaminomimetics in rats. Pol J Pharmacol 1997; 49:291-298