Apr 5th, 8:00 AM - 12:00 PM

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ABSTRACT

Attention deficit hyperactivity disorder (ADHD) is a neurobehavioral disorder that affects 11% of children in the US alone. Methylphenidate (MPH) is the most commonly prescribed drug for the treatment of ADHD. Given the fact that ADHD symptoms persist in up to 50% of patients, many children receive MPH from childhood to early adulthood. Unfortunately, most of the scientific literature focuses on the short-term consequences of MPH, even though individuals are taking MPH for many years. MPH acts by blocking dopamine (DA) transporters and norepinephrine transporters, preventing the reuptake of these catecholamines following release. Previous research has shown that long-term exposure to MPH causes dopaminergic neurons within the nigrostriatal pathway to be more sensitive to the Parkinsonian toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). We hypothesize that oxidative stress caused by the spontaneous oxidation of the excess DA in the synaptic cleft is what's rendering dopaminergic neurons within the nigrostriatal pathway to be more sensitive to MPTP. Adolescent male Swiss-Webster mice were divided into three cohorts and administered either saline (control), 1 mg/kg MPH (normal dose) or 10 mg/kg (abusive dose) via intraperitoneal (IP) injections for 12 weeks. Mice were injected twice daily, Monday through Friday, mimicking a school-week dosing schedule. After 12 weeks, all animals received a drug washout period of 7 days. Then, half of each cohort was treated with MPTP (4 x 20mg/kg, every 2 hours), while the other half was administered 4 injections of sterile saline. Seven days after MPTP or saline treatment, the mice were sacrificed, brains were removed, and the substantia nigra (SN) and striatum (STR) were collected. Oxidative stress related to increased DA levels was determined using the glutathione assay to measure glutathione (GSH) content and near-infrared fluorescence dot blots to measure free and protein-bound ortho-quinones. GSH is an important antioxidant and thus its depletion would be indicative of oxidative stress. Additionally, since DA may be oxidized to a quinone, increases in free and protein-bound ortho-quinones also indicate oxidative stress. Interestingly, we observed a significant decrease in GSH as the dose of MPH increased with both saline and MPTP samples. Furthermore, there was a significant increase in quinones as the dose of MPH increased. In conclusion, it appears that long-term exposure to MPH sensitizes dopaminergic neurons within the nigrostriatal pathway to oxidative stress, rendering them vulnerable to further insults, such as MPTP exposure. As such, these studies provide insight into the risks of long-term psychostimulant exposure.

INTRODUCTION

The primary mechanism of action of MPH is to prevent the reuptake of dopamine and norepinephrine by blocking pre-synaptic transporters on monoaminergic neurons (Fig. 1). Previous research has shown that long-term exposure to MPH causes dopaminergic neurons within the nigrostriatal pathway to be more sensitive to the Parkinsonian toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).

We hypothesize that oxidative stress caused by the spontaneous oxidation of the excess dopamine following long-term MPH treatment is rendering dopaminergic neurons within the nigrostriatal pathway to be more sensitive to MPTP.

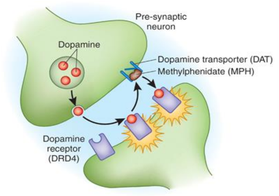


Figure 1. MPH blocks the reuptake of dopamine and norepinephrine, increasing the levels of these monoamines in the synaptic cleft (1).

By interfering with the electron transport chain, MPTP inhibits mitochondrial metabolism, causing a build up of free radicals and leading to dopaminergic cell death. The increase in oxidative stress and subsequent loss of dopaminergic neurons and induces Parkinsonian symptoms. During oxidative stress associated with elevated dopamine, quinone levels increase and glutathione levels decrease. Therefore, oxidative stress was measured using a glutathione assay and a near-infrared fluorescence dot blot (used to measure quinone production).

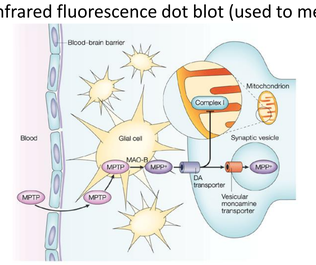
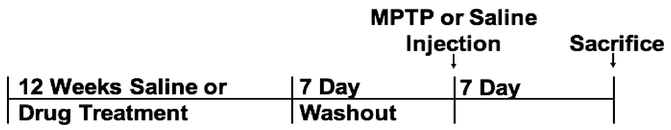


Figure 2. MPTP mechanism (2). MPTP targets and destroys dopaminergic neurons resulting in Parkinson's-like symptoms. MPTP crosses the blood-brain barrier where it is oxidized by MAO to MPP+ in glial cells. Glial cells release MPP+, and MPP+ is taken up by neurons through the dopamine transporter. MPP+ then inhibits complex I, leading to cell death.

MATERIALS AND METHODS

Purpose: To determine if MPTP susceptibility due to long-term exposure to MPH is correlated with oxidative stress in the substantia nigra and the striatum.



In our study, adolescent male Swiss-Webster mice were divided into three cohorts and administered either saline (control), 1 mg/kg MPH (normal dose) or 10 mg/kg (abusive dose) via intraperitoneal (IP) injections for 12 weeks. Mice were injected twice daily, Monday through Friday, mimicking a school-week dosing schedule. After 12 weeks, all animals received a drug washout period of 7 days. Then, half of each cohort was treated with MPTP (4 x 20 mg/kg, every 2 hours), while the other half was administered 4 injections of sterile saline. Seven days after MPTP or saline treatment, the mice were sacrificed, brains were removed, and the substantia nigra (SN) and striatum (STR) were collected. Oxidative stress related to increased DA levels was determined using the glutathione assay to measure glutathione (GSH) content and near-infrared fluorescence dot blots to measure free and protein-bound ortho-quinones.

INCREASED QUINONE FORMATION WITH CHRONIC MPH

Excess dopamine may be autooxidized into quinones which will contribute to oxidative stress and cause cellular damage. Thus, increases in free and protein-bound quinones should be seen when oxidative stress occurs.

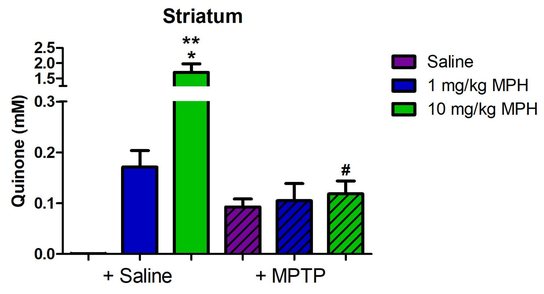


Figure 5. The amount of free and protein-bound quinones in the STR (n=4). One-way ANOVA followed by Fisher's post-hoc test.
*p<0.05 vs 1 mg/kg MPH + Saline; **p<0.05 vs Saline + Saline; #p<0.05 vs 10 mg/kg MPH + Saline

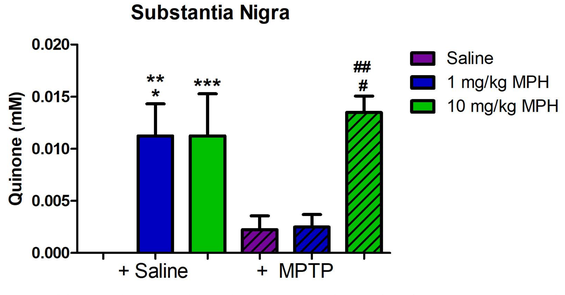


Figure 6. The amount of free and protein-bound quinones in the SN (n=4). One-way ANOVA followed by Fisher's post-hoc test .
*p<0.05 vs Saline + Saline; **p<0.05 vs 1 mg/kg MPH + MPTP; ***p<0.05 vs Saline + Saline; #p<0.05 vs 1 mg/kg MPH + MPTP; ##p<0.05 vs Saline + MPTP

GLUTATHIONE DEPLETION WITH CHRONIC MPH

Glutathione is an important antioxidant that protects against dopamine toxicity and thus its depletion would be indicative of oxidative stress.

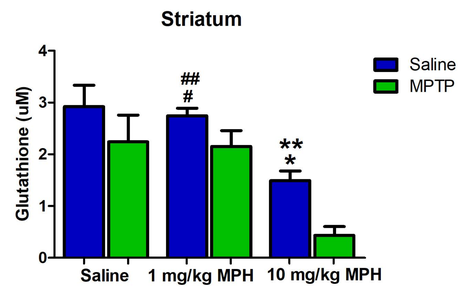


Figure 3. Glutathione content in the striatum (n=4). One-way ANOVA followed by Fisher's post-hoc test.

*p<0.05 vs Saline + Saline
**p<0.05 vs 10 mg/kg MPH + MPTP #p<0.05 vs 1 mg/kg MPH + MPTP
##p<0.05 vs 10 mg/kg MPH + Saline

CONCLUSIONS

There was a significant decrease in GSH seen in the striatum as the dose of MPH was increased with both saline and MPTP samples. Furthermore, there was a significant increase in quinones observed in the striatum and substantia nigra as the dose of MPH increased. In conclusion, it appears that long-term exposure to MPH sensitizes dopaminergic neurons within the nigrostriatal pathway to oxidative stress, rendering them vulnerable to further insults, such as MPTP exposure.

FUTURE DIRECTIONS

Previous studies investigating MPH use predominantly focus on the effect of MPH only in male subjects; little is known about the consequences of MPH exposure to females. Although estrogen has been shown to be protective against MPTP[3], females have been shown to be at a greater risk for developing neurodegenerative disorders such as Parkinson's disease with repeated exposure to other psychostimulants (amphetamines)[4]. Therefore, we are interested in determining the extent of oxidative stress within the nigrostriatal pathway of cycling females after long-term exposure to MPH. These experiments will provide insight into how chronic MPH affects dopaminergic neurons within the nigrostriatal pathway as well as insight into how estrogen influences the effects of chronic MPH in the female brain. **Our hypothesis is that long-term exposure to MPH will reduce the neuroprotective actions of estrogen against MPTP and sensitize the female animals to its neurotoxic effects.**

ACKNOWLEDGEMENTS

The authors would like to thank the Division of Laboratory Animal Services and Dr. Greg Hanley for help with our animals. This work was supported by a Research Development Committee Interdisciplinary grant, the American Foundation for Pharmaceutical Education (AFPE) and the department of Pharmaceutical Sciences at Bill Gatton College of Pharmacy.

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