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Research Report

Brain apoptosis signaling pathways are regulated by methylphenidate treatment in young and adult rats

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ABSTRACT

Methylphenidate (MPH) is commonly prescribed for children who have been diagnosed with attention deficit hyperactivity disorder (ADHD); however, the action mechanisms of methylphenidate have not been fully elucidated. Studies have shown a relationship between apoptosis signaling pathways and psychiatric disorders, as well as in therapeutic targets for such disorders. So, we investigated if chronic treatment with MPH at doses of 1, 2 and 10 mg/kg could alter the levels of pro-apoptotic protein, Bax, anti-apoptotic protein, Bcl-2, caspase-3 and cytochrome c in the brain of young and adult Wistar rats. Our results showed that MPH at all doses increased Bax in the cortex; the Bcl-2 and caspase-3 were increased with MPH (1 mg/kg) and were reduced with MPH (2 and 10 mg/kg); the cytochrome c was reduced in the cortex after treatment with MPH at all doses; in the cerebellum there was an increase of Bax with MPH at all doses, however, there was a reduction of Bcl-2, caspase-3, and cytochrome c with MPH (2 and 10 mg/kg); in the striatum the treatment with MPH (10 mg/kg) decreased caspase-3 and cytochrome c; treatment with MPH (2 and 10 mg/kg) increased Bax and decreased Bcl-2 in the hippocampus; and the caspase-3 and cytochrome c were reduced in the hippocampus with MPH (10 mg/kg). In conclusion, our results suggest that MPH influences plasticity in the brain of young and adult rats; however, the effects were dependent of age and brain area, on the one hand activating the initial cascade of apoptosis, increasing Bax and reducing Bcl-2, but otherwise inhibiting apoptosis by reduction of caspase-3 and cytochrome c.

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1. Introduction

Methylphenidate (MPH) is widely used for children who have been diagnosed with attention deficit hyperactivity disorder (ADHD) (Challman and Lipsky, 2000). However, MPH treatment is also used for other indications; for example, MPH presented efficacy for apathy in Alzheimer disease (Drye et al., 2013) and prevented bulimia nervosa (Guerdjikova and McElroy, 2013). It is known that MPH blocks the dopamine transporter and increases the levels of extracellular dopamine (Carboni et al., 2003), beyond inhibits the norepinephrine transporter (Madras et al., 2005). However, the MPH mechanism of action is not fully elucidated yet. There are also other studies that show the molecular effects of MPH. Indeed, MPH treatment altered circadian clock gene product expression (Baird et al., 2013) and N-methyl-D-aspartate (NMDA) receptor composition synaptic plasticity (Urban et al., 2013). In addition, MPH has been shown to influence energy metabolism and oxidative stress (Gomes et al., 2009; Réus et al., 2013). It is well known that those alterations could lead to activation of apoptotic pathways and cell death (Hyman and Yuan, 2012). In fact, initial activation of caspases can be from plasma membrane upon ligation of death receptor or from mitochondrial damage (Tekpli et al., 2013).

The mitochondrial apoptotic pathway is initiated by release of apoptogenic factors, for example, the cytochrome c, which activates caspase-3 and initiates the apoptotic process (Saelens et al., 2004). On the other hand, there are neuroprotective factors, such as the Bcl-2 protein, that protect the cells against mitochondrial damage and inhibit apoptosis (Martinou et al., 1994). More recently, studies have pointed out an important role of caspases for the activation of mediating neuronal cell death and neuronal loss in neurodegenerative diseases, as well as mood disorders (Cui et al., 2012; Dygalo et al., 2012). However, there are few reports about the effects of MPH in apoptosis signaling pathways. So, the present study was aimed to evaluate the effects of chronic administration of MPH in pro-apoptotic protein, Bax, anti-apoptotic protein, Bcl-2, caspase-3 and cytochrome c in the cortex, cerebellum, striatum and hippocampus of young and adult Wistar rats. These brain areas were chosen because they participate in circuits involved in ADHD (Volkow et al., 2002; Li et al., 2007).

2. Results

2.1. *Effects of MPH on Bax, Bcl-2, caspase-3 and cytochrome c in young rats*

The treatment with MPH at all doses increased Bax levels (Fig. 1A; $F_{(3-15)}=7.404$; $p=0.005$) in the cortex. The Bcl-2 (Fig. 1A; $F_{(3-15)}=38.811$; $p<0.001$) and caspase-3 levels (Fig. 1A; $F_{(3-15)}=27.134$; $p<0.001$) were increased with MPH at the dose of 1 mg/kg, and were reduced with MPH at the doses of 2 and 10 mg/kg. The cytochrome c was reduced in the cortex after treatment with MPH at all doses in young rats (Fig. 1A; $F_{(3-15)}=19.478$; $p<0.001$).

In the cerebellum there was an increase of Bax levels with MPH at all doses (Fig. 1B; $F_{(3-15)}=6.821$; $p=0.006$), however, there was a reduction of Bcl-2 (Fig. 1B; $F_{(3-15)}=57.894$; $p<0.001$), caspase-3 (Fig. 1B; $F_{(3-15)}=15.630$; $p<0.001$) and cytochrome c (Fig. 1B; $F_{(3-15)}=19.646$; $p<0.001$) levels with MPH at the doses of 2 and 10 mg/kg in young rats.

In the striatum the treatment with MPH at the dose of 10 mg/kg decreased caspase-3 (Fig. 1C; $F_{(3-15)}=7.187$; $p=0.005$) and cytochrome c (Fig. 1C; $F_{(3-15)}=16.696$; $p<0.001$) levels. On the other hand the Bax (Fig. 1C; $F_{(3-15)}=0.934$; $p=0.454$) and Bcl-2 (Fig. 1C; $F_{(3-15)}=1.290$; $p=0.322$) levels did not alter in the striatum.

Treatment with MPH at the doses of 2 and 10 mg/kg increased Bax (Fig. 1D; $F_{(3-15)}=7.693$; $p=0.004$) and decreased Bcl-2 (Fig. 1D; $F_{(3-15)}=95.118$; $p<0.001$) levels in the hippocampus. The caspase-3 (Fig. 1D; $F_{(3-15)}=7.678$; $p=0.004$) and cytochrome c (Fig. 1D; $F_{(3-15)}=22.807$; $p<0.001$) levels were reduced in the hippocampus with MPH at the dose of 10 mg/kg.

2.2. *Effects of MPH on Bax, Bcl-2, caspase-3 and cytochrome c in adult rats*

In the cortex of adult rats there was an increase of Bax levels with MPH at all doses (Fig. 2A; $F_{(3-15)}=27.396$; $p<0.001$). The Bcl-2 (Fig. 2A; $F_{(3-15)}=28.766$; $p<0.001$) and caspase-3 (Fig. 2A; $F_{(3-15)}=11.262$; $p=0.001$) levels were increased with MPH at the dose of 1 mg/kg and reduced with MPH at the doses of 2 and 10 mg/kg. The cytochrome c was reduced in the cortex with MPH at all doses (Fig. 2A; $F_{(3-15)}=134.562$; $p<0.001$).

The Bax levels were increased (Fig. 2B; $F_{(3-15)}=177.188$; $p<0.001$), and the cytochrome levels were reduced (Fig. 2B; $F_{(3-15)}=70.985$; $p<0.001$) in the cerebellum after treatment with MPH at all doses. Also, in the cerebellum the Bcl-2 (Fig. 2B; $F_{(3-15)}=58.029$; $p<0.001$) and caspase-3 (Fig. 2B; $F_{(3-15)}=74.549$; $p<0.001$) levels were reduced after treatment with MPH at the doses of 2 and 10 mg/kg in adult rats.

In the striatum there was an increase of Bax levels (Fig. 2C; $F_{(3-15)}=30.979$; $p<0.001$) and a reduction of caspase-3 (Fig. 2C; $F_{(3-15)}=34.305$; $p<0.001$). The Bcl-2 (Fig. 2C; $F_{(3-15)}=26.383$; $p<0.001$) and cytochrome c (Fig. 2C; $F_{(3-15)}=34.254$; $p<0.001$) levels were reduced in the striatum after treatment with MPH at the doses of 2 and 10 mg/kg.

In adult rats there was an increase of Bax (Fig. 2D; $F_{(3-15)}=23.476$; $p<0.001$) levels at all doses in the hippocampus. On the other hand, the Bcl-2 (Fig. 2D; $F_{(3-15)}=18.289$; $p<0.001$), caspase-3 (Fig. 2D; $F_{(3-15)}=18.632$; $p<0.001$) and cytochrome c (Fig. 2D; $F_{(3-15)}=69.707$; $p<0.001$) levels were reduced in the hippocampus after treatment with MPH at the doses of 2 and 10 mg/kg.

3. Discussion

It is well known that apoptosis signaling pathways are involved with neurodegenerative diseases and mood disorders, as well as therapeutic targets for such diseases (Leonard and Maes, 2012; Hu et al., 2013). However, few studies have reported the role of these pathways in ADHD or with the classic drug for the treatment of ADHD, MPH. As the use of