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Original research article

Methylphenidate increases glucose uptake in the brain of young and adult rats

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ABSTRACT

Background: Methylphenidate (MPH) is the drug of choice for pharmacological treatment of attention deficit hyperactivity disorder. Studies have pointed to the role of glucose and lactate as well as in the action mechanisms of drugs used to treat these neuropsychiatric diseases. Thus, this study aims to evaluate the effects of MPH administration on lactate release and glucose uptake in the brains of young and adult rats.
Methods: MPH (1.0, 2.0 and 10.0 mg/kg) or saline was injected in young and adult Wistar male rats either acutely (once) or chronically (once daily for 28 days). Then, the levels of lactate release and glucose uptake were assessed in the prefrontal cortex, hippocampus, striatum, cerebellum and cerebral cortex.
Results: Chronic MPH treatment increased glucose uptake at the dose of 10.0 mg/kg in the prefrontal cortex and striatum, and at the dose of 2.0 mg/kg in the cerebral cortex of young rats. In adult rats, an increase in glucose uptake was observed after acute administration of MPH at the dose of 10.0 mg/kg in the prefrontal cortex. After chronic treatment, there was an increase in glucose uptake with MPH doses of 2.0 and 10.0 mg/kg in the prefrontal cortex, and at an MPH dose of 2.0 mg/kg in the striatum of adult rats. The lactate release did not change with either acute or chronic treatments in young or adult rats.
Conclusions: These findings indicate that MPH increases glucose consumption in the brain, and that these changes are dependent on age and posology.

Introduction

Attention deficit hyperactivity disorder (ADHD) affects approximately 5% of children and 4% of adolescents and adults around the world [1]. The classic symptoms of this disorder are hyperactivity, impulsivity, and inattention. Although the etiology of ADHD is still uncertain, studies have shown genetic contributions to the disease's heritability [2]. The genes involved with the expression of the monoamine neurotransmitters dopamine, norepinephrine and serotonin, are most significantly associated with ADHD [3].

Treatment for ADHD is based primarily on psychotherapy and medications. Psychostimulant medications, such as the amphetamine drug methylphenidate (MPH), are most commonly used for pharmacologic treatment. MPH acts by blocking dopamine and norepinephrine reuptake transporters, thereby increasing the concentration of these neurotransmitters in the synaptic cleft [4]. Previous studies have demonstrated that MPH plays an important role in regulating energy metabolism in the brains of young and adult rats. In fact, MPH induced alterations in both Krebs cycle enzymes [5] and mitochondrial respiration [6–8]. Moreover, the mechanism of action of MPH has been attributed to regulation of brain apoptosis [9]. However, the mechanism of action of MPH in the brain still not fully understood.

Previous studies have shown that psychostimulant drugs, such as amphetamine, alter brain glucose metabolic rates, glucose

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uptake, and protect neurons against oxygen–glucose deprivation [10,11]. Lactate is another important fuel source in the brain. Moreover, lactate has been proposed as a target for new therapeutic drugs and interventions. In fact, lactate administration was able to increase systemic blood lactate levels, leading to both an increase in glucose and pyruvate and reduction in glutamate levels in the brains of injured patients [12]. These results are interesting, since lactate alone is not able to sustain normal brain function in adults because the capacity of the blood–brain barrier transporters for lactate is low [13]. Thus, glucose is the main energy source for the adult brain. In addition, the cerebral glucose metabolism may be influenced by mitochondrial dysfunction [14]. Since MPH influences energy metabolism, we hypothesize that MPH could influence brain glucose and lactate levels. To study this further, we conducted a study to evaluate the effects of treatment with MPH on lactate production and glucose consumption in the brains of young and adult rats.

Material and methods

Animals

Young (25 days old) and adult (60 days old) Wistar rats were housed five to a cage with food and water available *ad libitum* and were maintained on a 12-h light/dark cycle (lights on at 7:00 a.m.). The total number of young rats was 40 and adult rats was 40 for both acute and chronic treatments and all biochemical analyses. *In vivo* studies were performed in accordance with the National Institutes of Health guidelines and with the approval of the ethics committee from Universidade do Extremo Sul Catarinense (UNESC) under protocol number 35/2012.

Treatment

Acute treatment

Methylphenidate HCl (from Norvartis, Brazil) (1.0, 2.0 or 10.0 mg/kg, intraperitoneal) or saline injections (control group) were administered in young and adult rats at postnatal day (PD) 25 or PD 60 (6 animals per group), respectively according to previous studies [5,15–16].

Chronic treatment

Methylphenidate HCl (1.0, 2.0 or 10.0 mg/kg, intraperitoneal) or saline injections (control group) were administered in young rats starting on PD 25 once a day for 28 days (last injections on PD 53; 6 animals per group). The same treatment was given to adult rats starting on PD 60 once a day for 28 days (last injection on PD 88; 6 animals per group) according to previous studies [15,16].

Tissue preparation

After both protocols, acute and chronic, rats were sacrificed by decapitation 2 h after the last injection. Cerebellum, striatum, prefrontal cortex, hippocampus and cerebral cortex (total cortex without prefrontal cortex) were quickly isolated by hand dissection using a magnifying glass and a thin brush, based on the histological distinctions described by Paxinos and Watson [17]. Cerebellum, striatum, prefrontal cortex, hippocampus and cerebral cortex were cut into two perpendicular directions in order to produce 400 μm wide prisms using a McIlwain chopper. Prisms were pooled, weighed and used for lactate release and glucose uptake assays.

Biochemical analysis

Lactate release

Prefrontal cortex, hippocampus, striatum, cerebellum and cerebral cortex prisms (100 mg) were incubated in a metabolic shaker (90 oscillations min^{-1}) under a O_2/CO_2 (19:1) mixture at 37 °C for 60 min in Krebs–Ringer bicarbonate buffer, pH 7.0 (in a total volume of 1 ml). Two volumes of perchloric acid 0.6 N were immediately added to the prisms and the excess of perchloric acid was precipitated as a potassium salt by the addition of one volume of solution containing 0.5 N KOH, 0.1 M imidazol, and 0.1 M KCl [18]. After centrifugation for 5 min at $800 \times g$, lactate was measured in the supernatant before and after incubation by the lactase-peroxidase method, and determined routinely by commercial kit (BioTécnica, São Bernardo do Campo, Brazil) [19]. Lactate release was achieved by subtracting the amounts found after incubation from the amount found before incubation. Lactate levels in the medium at the beginning of the incubation were indetectable.

Glucose uptake

Prefrontal cortex, hippocampus, striatum, cerebellum and cerebral cortex prisms (100 mg) from the rats were incubated in Krebs–Ringer bicarbonate buffer, pH 7.0 (in a total volume of 1 ml), containing 5.0 mM glucose under an O_2/CO_2 (19:1) mixture in a metabolic shaker (90 oscillations min^{-1}) at 37 ◦C for 60 min [18]. Glucose was measured by the glucose oxidase method, and determined routinely by commercial kit (Labtest, Vista Alegre, Brazil) [20]. The uptake was determined by subtracting the amount after incubation from the total amount measured before incubation.

Statistical analysis

All data are presented as mean ± standard deviation. Differences among experimental groups in the assessment of lactate release and glucose uptake were determined by one-way ANOVA, followed by Tukey *post hoc* test when ANOVA was significant; p values < 0.05 were considered to be statistical significant.

Results

Effects of acute and chronic treatment with MPH in glucose uptake and lactate release in young rats

Fig. 1 shows the effects of MPH treatment in glucose uptake in the brain of young rats. It can be seen that acute treatment with MPH did not alter glucose uptake in the prefrontal cortex ($F_{(3-13)} = 1.522$; $p = 0.269$; Fig. 1A), cerebellum ($F_{(3-15)} = 1.243$; $p = 0.337$; Fig. 1A), hippocampus ($F_{(3-15)} = 0.971$; $p = 0.438$; Fig. 1A), striatum ($F_{(3-14)} = 1.290$; $p = 0.326$; Fig. 1A) and cerebral cortex ($F_{(3-14)} = 3.293$; $p = 0.062$; Fig. 1A). On the other hand, after chronic treatment there was an increase in glucose uptake with an MPH dose of 10.0 mg/kg in the prefrontal cortex ($F_{(3-19)} = 6.046$; $p = 0.006$; Fig. 1B) and in the striatum ($F_{(3-18)} = 11.717$; $p < 0.001$; Fig. 1B). With an MPH dose of 2.0 mg/kg, glucose uptake also increased in the cerebral cortex ($F_{(3-13)} = 9.471$; $p = 0.001$; Fig. 1B). However, in the hippocampus ($F_{(3-18)} = 2.423$; $p = 0.106$; Fig. 1B) and cerebellum ($F_{(3-19)} = 2.095$; $p = 0.141$; Fig. 1B) there were no changes after chronic treatment with any dose of MPH in glucose uptake. Neither acute nor chronic treatments were able to alter lactate release in the brains of young rats (Fig. 2A and B).

Effects of acute and chronic treatment with MPH in glucose uptake and lactate release in adult rats

In adult rats, the acute treatment with MPH at the dose of 10.0 mg/kg increased glucose uptake in the prefrontal cortex ($F_{(3-17)} = 5.445$; $p = 0.011$; Fig. 3A). However, at this dose, we did not observe changes in the cerebellum ($F_{(3-18)} = 2.033$; $p = 0.152$; Fig. 3A), hippocampus ($F_{(3-15)} = 1.720$; $p = 0.216$; Fig. 3A), striatum