Original Article

METHYLPHENIDATE: PROCONVULSANT EFFECT AND ACTION ON ACETYL CHOLINESTERASE ACTIVITY IN YOUNG AND ADULT MICE

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ABSTRACT

Objective: Methylphenidate (MPH) is a derivative of piperidine, structurally related to amphetamine. It is the most widely psychostimulant used in Brazil for treating attention deficit disorder and hyperactivity. This drug was investigated in an epilepsy model induced by pilocarpine and on acetylcholinesterase (AChE) activity in young and adult mice.

Methods: The control group was treated with saline. The treated groups received MPH (2.5, 5, 10 or 20 mg/kg) in single dose, followed by pilocarpine (400mg/Kg). The groups were observed for1h after treatment.

Results: MPH, in all doses, was efficacious in decreasing both the latency to first seizures and the survival percentage in young and adult animals. Determination of AChE activity in the hippocampus and striatum of young and adult animals, after pilocarpine-induced status epilepticus, demonstrated that pretreatment with MPH reduced AChE activity only in the striatum.

Conclusion: Our findings suggest that MPH has proconvulsant action and cholinergic neurotransmission system can play a role in this effect.

Keywords: Acetylcholinesterase, Hippocampus, Methylphenidate, Seizures, Striatum.

INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) is the most commonly diagnosed neuropsychiatric disorder in childhood, characterized by excessive levels of inattentiveness, impulsivity, and hyperactivity. The prevalence is variable but has been estimated at 3-9% of school-aged children and 4% of adults [1, 2, 3]. Most ADHD patients benefit from treatment with methylphenidate hydrochloride (MPH), irrespective of the etiology of the disorder, effectively reducing symptoms in up to 70% of children [4]. However, recognition that ADHD persists into adulthood has led to increased use of MPH in adult patients [5].

Patients with ADHD exhibit dysfunction of dopaminergic and noradrenergic circuits in the brain, including the prefrontal and subcortical regions (e.g., striatum), and limbic regions (e.g., hippocampus) [6, 7, 8, 9].

Biochemical studies have shown that MPH, like amphetamine, enhances the release and blocks the reuptake of noradrenaline and dopamine in mammalian brain [10, 11, 12]. After administration, the distribution of MPH in brain is heterogeneous, and the maximum concentration occurs in the striatum, cortex, and cerebellum [12].

The acute administration of high doses of pilocarpine, a muscarinic cholinergic agonist, induces behavioural changes and seizures which progress after 1–2 h to long-lasting status epilepticus (SE) [13]. The neurochemical and behavioural characteristics of pilocarpine induced epilepsy in rodent models appear to be similar to those observed in human temporal lobe epilepsy [14]. Other studies suggest permanent changes in other neurochemical systems t such as the cholinergic system might be altered after seizures and SE induced by pilocarpine [15,16]. Therefore, we considered it important to study the enzymatic activity of acetylcholinesterase related to cholinergic system during seizures.

Acetylcholinesterase (AChE) hydrolyzes the neurotransmitter acetylcholine (ACh) at the synaptic cleft of cholinergic synapses and neuromuscular junctions [17]. ACh has also been demonstrated to up- and down-regulate the development and differentiation of neural cells [18]. Increased ACh levels can dramatically alter neuronal function and excessive release and increased synthesis of this neurotransmitter has been related to SE [19]. In particular, it has been suggested that ACh, acting via intracellular messengers, may be involved in adult neuronal plasticity [20] and that an excess of ACh can induce SE in young and adult rats [15].

The aim of the present study was to investigate the effect of acute administration of MPH on cholinergic model of seizures induced by pilocarpine, as well as to determine the AChE activity in hippocampus and striatum of young and adult mice treated with MPH after pilocarpine-induced SE.

MATERIAL AND METHODS

Swiss, male mice young (8-15 g; 21 days old) and adult (25-30 g; 2 months old) were obtained from Central Animal House of the Federal University of Ceara (UFC), Ceara, Brazil. Animals were housed in cages with free access to food and water. All animals were kept with standard light–dark cycle (lights on at 07:00 h a.m.). Experiments were performed under the consent and surveillance of Ethics Committee on Animal Research of UFC (CEPA; protocol number: 09/2012).

Methylphenidate-Ritalin® was obtained commercially (Novartis Laboratorie). MPH was dissolved in distilled water. Pilocarpine hydrochloride (400 mg/kg (P400); (Sigma, Chemical USA) was administered in a volume of 10mL/kg injected intraperitoneally (i.p.).

The animals were divided into six groups of 10 mice (n=10) and treated with MPH administered at doses of 2,5mg/kg (MPH 2,5); 5 mg/kg (MPH 5); 10 mg/kg (MPH 10) and 20 mg/kg (MPH 20), intragastrically and, after 60 min, with (cholinergic agonist), 400 mg/kg (P400), injected intraperitoneally (i.p.). One group received only P400. Each animal was placed in the acrylic observation

chamber (107mm×202mm×150 mm) immediately after injection of P400, and the occurrence of clonic convulsions and mortality, as well behavioural changes were observed for 1h. After the SE, the animals that survived to P400 treatment (groups pretreated with MPH) and / or a control group of 0.9% saline (group only used to measure the enzyme AChE) were sacrificed by decapitation 1 h after treatment and their brains were dissected on an ice cold plate to remove cerebral areas (striatum and hippocampus), then weighed and stored at -70 ∘ C. Tissues were ultrasonically homogenized in 1 ml of 0.05 M phosphate buffer, pH 7.0 to measure the activity of AChE.

AChE activity was measured by the method of Ellman et al. [21] using 1mM acetylthiocholine, 1mM 5,5- dithiobis-(2-nitrobenzoic acid) and an incubation period of 10 min at 37 °C. The results were expressed as nmol acetylthiocholine hydrolyzed/min/mg protein. Results of latency to first seizure and neurochemical alterations were compared by one-way analysis of variance (ANOVA) followed by t Student–Newman–Keuls as post hoc test (P < 0.05) (Graphpad program Intuitive, Software for Science, San Diego, CA, USA). The number of animals that had seizures and the number that survived were expressed as percentages (latency of convulsion and survival percentage).

RESULTS AND DISCUSSION

A few minutes after the administration of P400, the animals showed peripheral cholinergic signs (100%) (miosis, piloerection, tear of blood, salivation, diarrhea, dieresis, tremors) and stereotyped movements (increased activity of biting, scratching, chewing and wet dog shakes - shaking like a wet dog), followed by limbic motor seizures. These changes were more pronounced in the pre-treated with MPH. Observing all animals, we found that the latency of convulsions was significantly decreased in the groups pretreated with MPH (MPH2,5+P400; MPH5+P400; MPH10+P400; MPH20+P400) when compared with the P400 group in young mice [P400 = 538,7±39,63 (n=13); MPH2,5+P400 = 493,7±34,56 (n=13); MPH5+P400 = 423,8±25,63 (n=13); MPH10+P400 = 378,2±22,41 (n=15); MPH20+P400 = 357,2±25,28 (n=15)] (Figure 1A).
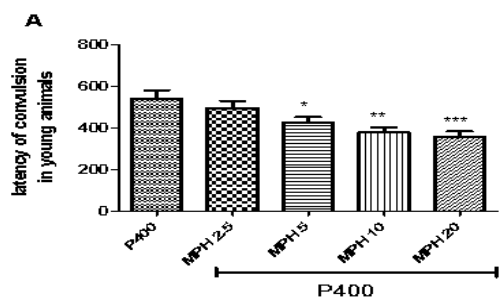
There was also a decrease in adult mice in the latency of seizures in animals pretreated with MPH when compared to the P400 [P400 = 875,7±42,80 (n=13); MPH2,5+P400 = 564,3±44,76 (n=13); MPH5+P400 = 632,3±46,90 (n=13); MPH10+P400 = 677,9±43,54 (n=15); MPH20+P400 = 696,0±25,99 (n=15)] (Figure 1B).
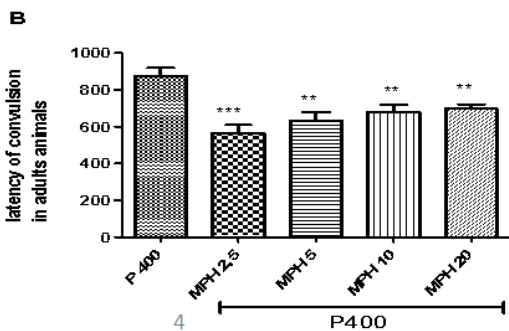

Fig. 1: It shows the latency of seizures (in seconds) in animal groups pretreated with Methylphenidate (MPH: 2,5; 5; 10 and 20 mg/kg) and after with Pilocarpine (P400mg/kg).

Where, A. Young animals and B. Adult animals. Data represent mean ± SEM. *P<0,05; **P<0,01 and ***P<0,001 compared to P400 alone (control group) (ANOVA and Student- Newman- Keuls).

In young animals there was a decrease in the percentage of survival in the group pretreated with MPH at all doses, where 100 % [MPH2,5+P400 (n=13); MPH5+P400 (n=13); MPH10+P400 (n=15); MPH20+P400 (n=15)] of animals died when compared to the P400 group (76,9%;10/13 of the animals died) over the time period of the experiment **(Figure 2A)**.

In adult animals, it occurred a decreased in the percentage of survival at all doses, where 100% of the animals died [(MPH2,5+P400 (n=8); MPH5+P400 (n=8); MPH10+P400 (n=14); MPH20+P400 (n=12)] compared to P400 group, where 64,28% (9/14) of the animal died over the time period of the experiment **(**Figure 2B).
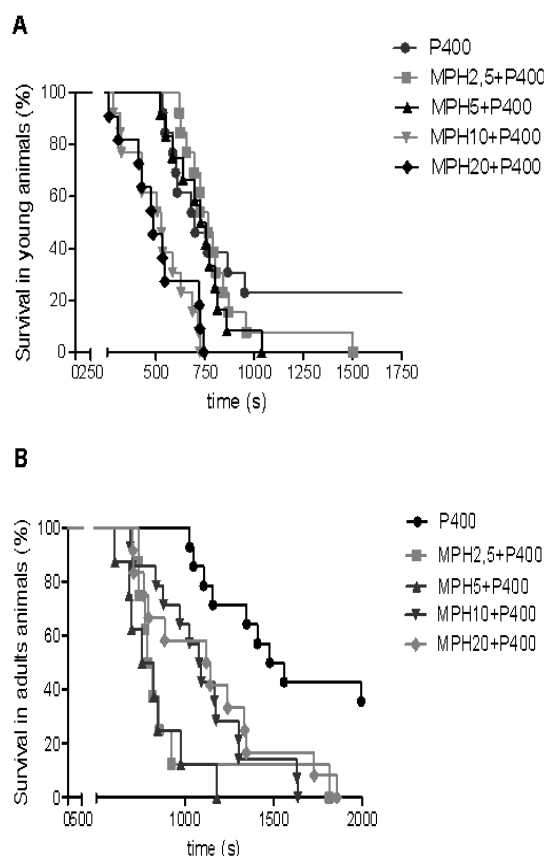

Fig. 2: It hows the effect of methylphenidate on percentage of survival of animal groups pretreated with Methylphenidate (MPH: 2,5; 5; 10 and 20 mg/kg) and after with Pilocarpine (P400mg/kg).

Where, A. Young animals and B. Adult animals

The investigation of the enzymatic activity of AChE was performed in hippocampus and striatum of young and adult mice, pertaining to the following treatment groups: CTRL (saline 0,9%); methylphenidate (MPH2,5+P400; MPH5+P400; MPH10+P400; MPH20+P400); pilocarpine, 400mg/Kg (P400). The results were expressed as nmol/mg protein/ minute.

In young animals: The administration only of P400 was able to significantly reduce the AChE activity in the hippocampus and striatum as compared to CTRL. No significant change was observed in the hippocampus of animals belonging to different groups of MPH pretreatment followed by P400 compared to CTRL and P400 [CTRL = 49,41±3,64 (n=6); P400 = 25,55+2,31 (n=8); MPH2,5+P400 = 42,19±7,36 (n=6); MPH5+P400 = 42,25±5,11 (n=6); MPH10+P400 = 53,28±4,50 (n=6); MPH20+P400 = 33,16±3,86 (n=6)] **(Figure 3A)**.

However, pretreatment with MPH followed by administration of P400 (MPH5+P400; MPH10+P400; MPH20+P400) reduced significantly the level of AChE enzyme activity in the striatum compared to CTRL [CTRL = 83,54±5,14 (n=7); P400 = 42,75±9,00 (n=6); MPH2,5+P400 = 67,10±5,37 (n=8); MPH5±P400 = 57,40±6,43 (n=7); MPH10±P400 = 43,03±7,60 (n=7); MPH20±P400 = 59,38±5,54 (n=6)] **(Figure 4A)**.

In adult animals: The administration of P400 was able to reduce significantly AChE activity in the hippocampus and striatum compared to CTRL. No significant change was observed in the hippocampus of animals belonging to different groups of MPH pretreatment followed by P400 compared to CTRL and P400 [CTRL = 63,06±7,10 (n=9); P400 = 19,04±2,80 (n=8); MPH2,5+P400 = 45,40±4,02 (n=9); MPH5+P400 = 40,61±5,99 (n=8); MPH10+P400 = 69,84±5,23 (n=8); MPH20+P400 = 54,72±7,76 (n=7)] **(Figure 3B)**. In the striatum, the groups pretreated with MPH followed by administration of P400 (MPH2,5+P400 and MPH5+P400) reduced significantly the level of AChE enzyme activity when compared to CTRL [CTRL = 141,8±12,71 (n=8); P400 = 48,76±4,70 (n=8); MPH2,5+P400 = 64,48±4,34 (n=9); MPH5+P400 = 56,50±5,30 (n=10); MPH10+P400 = 136,7±17,70 (n=6); MPH20+P400 = 143,0±16,60 (n=8)] (Figure 4B).
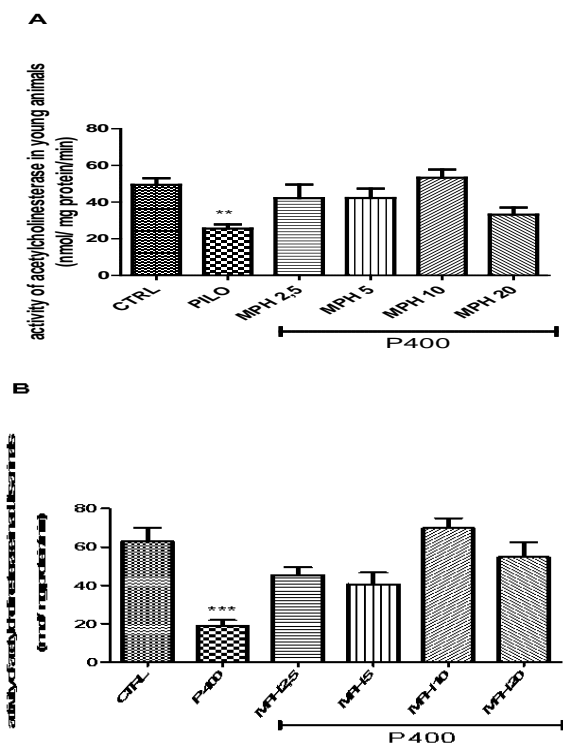

Fig. 3: It shows the effects of methylphenidate on the activity of acetylcholinesterase (AChE) in the hippocampus during seizures induced by Pilocarpine 400 mg/kg (P400).

Where, A. Young animals and B. Adult animals. Data represent mean± SEM. **P<0,01 and ***P<0,001 compared to control group (ANOVA and Student-Keuls).

Studies have shown high rates of neuropsychiatric comorbidities associated with ADHD [22, 23, 24]. A consensus of international experts emphasized the message that "comorbidity is the norm rather than exception" [23]. Souza et al [24] studied a group of children and adolescents, and found that more than 85% had comorbid disorders. Conduct disorders and oppositional defiance were the most common.. Other studies also indicate the presence of anxiety disorders, depression, bipolar disorder and motor tics [25]. ADHD symptoms can often precede the first recognized seizures or can become more apparent during the course of the epilepsy as the patient has repeated seizures [26, 22].
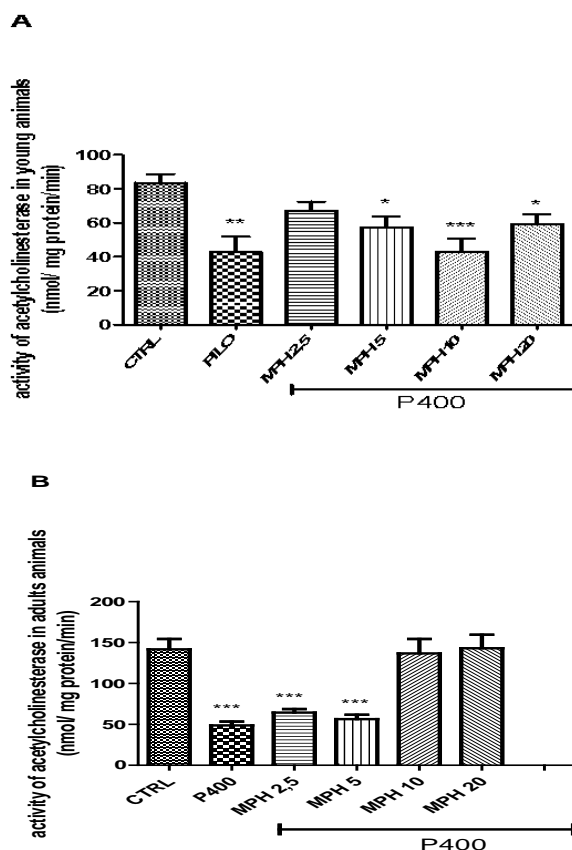

Fig. 4: It shows the effects of methylphenidate on the activity of acetylcholinesterase (AChE) in the hippocampus during seizures induced by Pilocarpine 400 mg/kg (P400)

Where, A. Young animals and B. Adult animals. Data represent mean± SEM. *P<0,05; **P<0,01 and ***P<0,001 compared to control group (ANOVA and Student-Keuls).

There is a warning in the FDA-approved package insert for all MPH products advising against use of MPH in patients with a history of seizures. However, the information to support this warning is very limited and contradicted by other lines of evidence [27].

MPH has been tested in a model of seizures induced by sensitization with low-intensity brain stimulation (kindling). In this model, MPH prolonged the duration of kindled seizures, but only weakly [28]. Overdosing on MPH may lead to stimulation of the central nervous system or sympathomimetic system, causing severe manifestations, including seizures [29]. The results of this study show that the seizures in the animals pretreated with MPH were potentiated, shown by reduced latency periods to onset of first seizure and time to death following administration of P400. This effect was observed in both young and adult animals.

The investigation of the putative mechanism involving the cholinergic system, was performed using pilocarpine, a muscarinic agonist, which in high doses induces behavioral changes, such as seizures and brain damage in rodents via cortical overstimulation [30,14]. The reduction of the latency period with MPH pro-treatment for seizures and survival suggest that MPH is involved in cholinergic pathways and does not protect against seizures in cholinergic models.

To further elucidate the role of cholinergic transmission in convulsions and death observed with MPH, the brain AChE activity of the animals was determined. Pilocarpine exacerbates cholinergic activity, probably due to direct influence, increasing the ACh current, modifying the binding of muscarinic receptors [31] and decreasing the activity AChE [32]. AChE is considered essential for normal functioning of the nervous system, as it limits the action of

acetylcholine, as soon as it is released in the synaptic clefts. It is speculated that the enzyme activity could affect the susceptibility to seizures induced by pilocarpine [33].

The inhibition of AChE activity induced by P400, in seizure models, has been previously reported [14, 34]. In the present study, consistent with the current literature, P400 induced a reduction in activity of this enzyme. Pre-treatment with MPH in young and adults animals reduced the enzyme activity in the striatum when compared to the control group. There were no significant differences in the hippocampus of young and adult animals pretreated with MPH. It has been previously described that treatment with MPH only increases the activity of AChE in the striatum but not in the hippocampus of rats [35].

Cholinergic activation is essential for the initiation of the seizures models in temporal lobe epilepsy, since these fits can be blocked by pretreatment with the antagonist atropine [36]. It is believed that the decrease of acetylcholine metabolism by reducing or blocking the activity of AChE, can facilitate installation of seizure activity, by virtue of increasing concentration of endougenous acetylcholine, which can directly activate the cholinergic system and, directly or indirectly, induce neurochemical changes in other neurotransmitter systems, including, glutamatergic and GABAergic, since these may be implicated in the establishment and development of limbic seizures [31]. Our data suggest that the mechanism of seizures caused by MPH may involve a modulation in the cholinergic system, with possible reduction of the activity of acetylcholinesterase, resulting in increased levels of acetylcholine [37].

CONCLUSION

In summary, this study suggests a modulating effect, exerted by MPH, on the functioning of the muscarinic cholinergic system, in the central level, as an alternative mechanism for potentiation of seizures in the P400 model, suggesting a possible cholinergic mechanism involved, since the drug reduced the activity of acetylcholinesterase.

ACKNOWLEDGMENT

CONFLICT OF INTEREST

This research has no conflict of interest.

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