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Original Article

ESTUDO DO L-TRIPTOFANO NA DEPRESSÃO OCORRIDA PELA DOENÇA DE ALZHEIMER EM MODELO EXPERIMENTAL

STUDY OF L-TRYPTOPHAN IN AN EXPERIMENTAL MODEL OF DEPRESSION CAUSED BY ALZHEIMER'S DISEASE

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ABSTRACT

Alzheimer's is a neurodegenerative disease characterized by cognitive impairment commonly associated with mood disorders, which trigger depressive reactions and compromise mental performance and functionality. The objective of the present study was to verify the effects of L-tryptophan and to analyze motor behavior in an experimental model of depression caused by the Alzheimer's disease process. The sample consisted of 40 Wistar rats divided equally into two groups, 20 animals treated with L-tryptophan and 20 control animals. Both groups received spatial memory training in the water maze task and were submitted to stereotaxic surgery to induce dementia. The treated group achieved better spatial memory activity than the control group in the Morris water maze. Treatment with L-tryptophan had beneficial effects on reactive memory. **Keywords:** Alzheimer's Disease. Depression. Serotonin. Tryptophan.

RESUMO

O Alzheimer é uma doença neurodegenerativa caracterizado pelo comprometimento cognitivo comumente associado a transtornos do humor, os quais desencadeiam reações depressivas, comprometem o desempenho mental e a funcionalidade. O objetivo do presente estudo foi verificar os efeitos do L-triptofano e analisar o comportamento motor em modelo experimental com depressão decorrente do processo de Alzheimer. A amostra foi composta por 40 ratos da linhagem *wistar* divididos igualmente em dois grupos, 20 animais tratados com L-triptofano e 20 animais pertencentes ao grupo controle. Ambos os grupos receberam treinamento da memória espacial no *later water maze* e foram submetidos à cirurgia estereotáxica para indução demencial. Verificou-se através do labirinto aquático de *Morris* que o grupo tratado obteve atividade para memória espacial melhor do que o grupo controle. O tratamento com L-triptofano demonstrou melhor benefício na memória reativa.

Palavras-chave: Doença de Alzheimer. Depressão. Serotonina. Triptofano.

Introduction

Alzheimer's disease (AD) is a slowly developing and gradually progressing degenerative neurological disease that first affects the episodic memory and is more common after the fifth decade of life[1,2]. This type of dementia accounts for 60% of all cases of progressive cognitive impairment in older adults[3]. Affected patients manifest difficulties in the acquisition of new tasks, as well as difficulties remembering, deciding, reacting and eating. In a more advanced stage, the patient enters a vegetative state, including alterations in circadian cycle, behavioral changes, psychotic symptoms, and the inability to walk, talk and perform self-care[2,4].

The mechanisms of neurodegeneration first reach the structures of the medial temporal lobe, including the hippocampus and parahippocampal gyrus which are important for memory. Degeneration subsequently affects other regions of the associative neocortex, compromising cognition. This degeneration is due to the abnormal formation of senile plaques and neurofibrillary tangles as a result of a modification in the amyloid precursor

protein and to hyper-collapse of the neuronal cytoskeleton caused by hyperphosphorylation of tau protein[5]. Neuronal degeneration is the result of a cascade process including dysregulation of cholinergic neurons, synaptic decay, neuroinflammation, autophagy and apoptosis, abnormalities that cause changes in the behavior and performance of the individual with AD[6].

The early identification of dementia may improve the quality of life of the affected individual and minimize cognitive decline[7-9], as well as provide a better prognosis since AD is commonly associated with mood disorders that can trigger depressive reactions and compromise mental performance and functionality[10-12].

Depression is a mood disorder characterized by biochemical changes in the brain that result from a decline in the metabolism of serotonin, the main neurotransmitter responsible for a balanced mood and sensation of well-being[12]. Glucocorticoids or corticosteroids may also be involved in depression since their excess restricts dopamine levels[13,14].

Serotonin or 5-hydroxytryptamine (5-HT) is synthesized in neurons of brainstem raphe nuclei by hydroxylation and decarboxylation of L-tryptophan. The latter is an essential amino acid and is considered the only precursor of 5-HT that is produced both in the central nervous system (CNS) and peripherally. In addition, L-tryptophan is involved in the formation of proteins and metabolites such as kynurenine[15-17]. Through a diffuse network within the CNS, the serotoninergic system is responsible for regulating different functions such as sleep, appetite, temperature, mood, and cognition[16].

Behavioral and psychological changes arise with the progression of AD and become the reasons for institutionalization, medication use, increased costs of care with dementia, and family burden[18]. In addition, neurophysiological and neurochemical changes interfere with mood and cause depressive symptoms that are responsible for increased morbidity and mortality, a growing use of health services, self-care neglect, reduced adherence to treatment, and higher suicide rates[10,19]. Thus, studies investigating psychological disorders related to AD are necessary to increase our understanding of the mechanisms underlying these alterations and to elaborate pharmacological interventions that are able to delay the progression of the disease and its deleterious effects.

The objective of the present study was to verify the effects of L-tryptophan on depression caused by the Alzheimer's process and to analyze motor behavior in an animal model of AD.

Methods

Sample

The sample consisted of 40 Wistar rats (*Rattus norvegicus*) weighing 200 g that were purchased from the Animal House of Universidade Católica do Paraná (PUCPR) after approval by the Ethics Committee on Animal Use of CEUA/UNICENTRO (Protocol No. 020/2015). The procedures were carried out at the Laboratory of Neuroanatomy and Neurophysiology of UNICENTRO. In the laboratory, the animals were kept in cages on rack shelves (4 animals per cage) with free access to water and ration under a 12-h light/dark cycle (lights on from 7:00 to 19:00 h) at a temperature of 23±1ºC controlled by a sprint air conditioning unit (7000 BTUs).

Table 1. General data of the animals

Number	Weight	Species	Lineage	Age
40 rats	± 200 g	*Rattus norvegicus*	Wistar	2 months term

Experimental groups

The animals were divided into two groups:

Control group (CG): composed of 20 animals with lesion in the CA1 area (Alzheimer's) not submitted to treatment. The animals were euthanized 21 days after surgery.

Group treated with L-tryptophan (TG): composed of 20 animals with lesion in the CA1 area (Alzheimer's) and treated orally with 100 mg/kg L-tryptophan dissolved in 0.9% saline for 21 days. The animals were euthanized after L-tryptophan treatment.

Experimental surgery

The animals were anesthetized by intra-abdominal injection of a solution of 80 mg/kg ketamine hydrochloride (Ketamina, 10-ml flask) and 15 mg/kg xylazine hydrochloride (Dopaser, 10-ml flask) and transferred to a stereotaxic instrument (David Kopf, USA), where their heads were secured by the temporal bone and upper incisors. Cannulae with a 5-mm needle were implanted bilaterally into the hippocampus and directed towards the hippocampal CA1 area according to the following coordinates: anteroposterior = -3.0 mm, mediolateral = ± 1.6 mm and -1.6 mm, and dorsoventral = 3.0 mm from the bregma. The lambdoid and bregmatic sutures were in the same horizontal plane according to the Atlas of Paxinos and Watson[20]. After implantation, the cannulae were fixed in the calvaria with a self-curing acrylic prosthesis. A stainless-steel wire was inserted to prevent occlusion of the cannula. The wire was fixed by placing a screw in the anterior portion of the skullcap. For analgesia, the animals received orally 10 mg/kg tramadol hydrochloride diluted in 50 ml water every 12 hours for 7 days[21].

The animals were allowed to rest for 5 days. After this period, the animals were again anesthetized and transferred to the stereotaxic instrument. Beta-amyloid$_{25-35}$ peptide (Sigma-Aldrich) was injected with a Hamilton syringe into the hippocampal CA1 region as described by Freir, Costello and Herron[22].

Analysis of motor behavior

Open-field test protocol

The animal was placed in the center of the arena and exposed individually to the open field for a period of 5 minutes during which the behaviors were recorded with a video camera. For ethological analysis of the behaviors, the frequency and duration of walking, rearing and grooming were determined, as well as the number of fecal boli. Walking was measured by the number of rectangles invaded with the four paws. Rearing only considered standing on the hind legs. Grooming was evaluated by movements directed at the head or body, performed with the front legs. Fecal boli were counted after removal of the animal from the arena. Between exposure of one rat and the other, the floor of the arena was cleaned with alcohol and dried and air was allowed to circulate.

Morris water maze (MWM)

The MWM consists of a circular pool made of polypropylene fiber (200 cm in diameter and 50 cm deep), which rests on a wooden frame. The inner walls are painted black to ensure homogeneity. The water temperature is about 26±1°C.

For the test, four starting points were established from the border of the pool: north, south, east, and west. The center of the quadrant of the maze delimited by these cardinal points contains a socket at the bottom of the pool that permits fixation of a platform. Once in place, the platform remains approximately 2 cm below the water level, invisible to the rat. The platform is made of clear acrylic and measures 11 x 14 cm so that the animal can escape from water onto the platform (aversion). Visual clues consisting of geometric figures and drawings were placed on the walls of the room around the pool to serve as external reference points for localization of the animal in the pool.

The rats were trained in a spatial version of the water maze task. The animal was released randomly at one of the starting points, forcing it to orient itself based on the spatial relationships between clues to find the platform that remained in the same place throughout the experiment. Each animal was trained five times per day for 10 days before the induction of Alzheimer's and was tested once after treatment with L-tryptophan. The animal was allowed a maximum of 5 minutes during each attempt to find the center of the maze. If the animal did not find the platform, the location was indicated.

Statistical analysis

The data were entered into spreadsheets and analyzed with the GraphPad Prism 5.01 program. The Kruskal-Wallis test and Dunn's post test were used for analysis.

Results

Figure 1 shows the results of displacement in the arena obtained for treated and control animals before the induction of Alzheimer's (CG-A; TG-A), after the induction of dementia (CG-B; TG-B), and after treatment with L-tryptophan (CG-C; TG-C). The mean values were 715, 232.9, 298.4, 686.9, 134.6 and 355.7 cm in CG-A, CG-B, CG-C, TG-A, TG-B and TG-C, respectively. Displacement in the open field was reduced in CG and TG after the induction of AD, demonstrating that active beta-amyloid peptide alters the motor behavior of the animals, which are in a state of stagnation such as a depressive state. However, marked motor displacement was observed after treatment, which was higher when compared to CG. This finding demonstrates the effectiveness of L-tryptophan and its effect on the behavioral performance of treated anmals. However, intergroup comparison by the Kruskal-Wallis test and Dunn's post test ($p=0.1017$) did not reveal a significant difference.

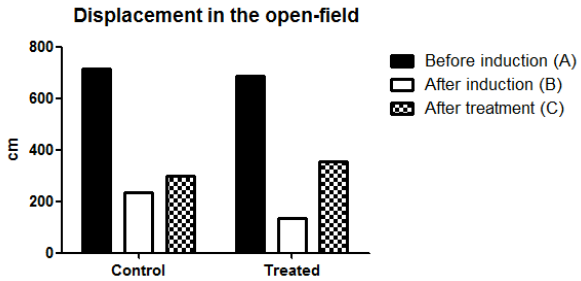

Figure 1. Mean displacement in the open-field arena of treated and control animals before and after induction of Alzheimer's disease and after treatment with L-trytophan.
Source: The authors.

Figure 2 shows the mean values and standard deviation in the MWM obtained for TG and CG before and after the induction of Alzheimer's. The mean TG-pre, CG-pre, TG-post and CG-post were 10.53, 9.903, 4.20 and 4.378 s, respectively. Intergroup comparison by the

Kruskal-Wallis test revealed p=0.0001. Significant differences were obtained by Dunn's post test between TG-pre/TG-post, TG-pre/CG-post, CG-pre/CG-post, and CG-pre/TG-post. A reduction in time was observed between TG-pre/TG-post, suggesting that L-tryptophan was effective in improving spatial memory in these animals.

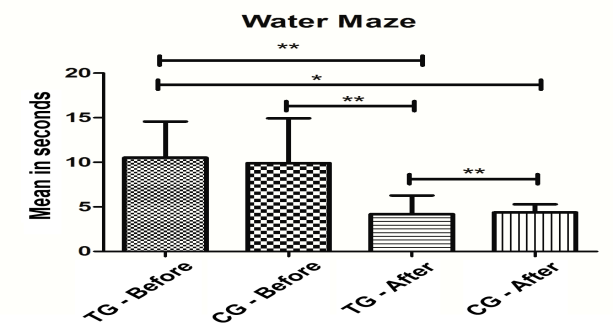

Figure 2. Mean values and standard deviation obtained for control (CG) and treated (TG) animals in the Morris water maze before and after induction of Alzheimer's.
* < 0.01; ** < 0.001
Source: The authors

Discussion

Glikmann-Johnston et al.[23] reported that pharmacological changes in 5-HT concentrations due to reuptake or release interfere with spatial memory function. In this respect, an increase in extracellular 5-HT concentration maintains or improves performance, while a reduction is detrimental to spatial memory.

In the present study, the animals ingested L-tryptophan at a dose of 100 mg/kg diluted in 0.9% saline for 21 days in order to stimulate serotonin synthesis. Animals of TG and CG were trained for 10 days in the MWM before the induction of Alzheimer's and both groups rapidly found the central quadrant in the maze already after the second day of training. However, after the induction of dementia, TG animal performed the task more easily and in less time than CG animals. These findings confirm the effectiveness of L-tryptophan in improving reactive memory, behavioral state and spatial orientation of the animals.

Cai et al.[24], Remondes and Schuman[25] and Cai et al.[26] demonstrated the potentiation of excitatory synapses by endogenous 5-HT in the anterior brain, more precisely in areas CA1 and CA3 of the hippocampus. This serotoninergic pathway is fundamental for cognitive tasks, spatial recognition, spatial memory, and long-term memory consolidation. Furthermore, increased levels of 5-HT induced by drugs can modulate neuronal plasticity and excitability. A study on rodents has shown that 5-HT intensifies postsynaptic transmission and is altered in experimental models of depression.

In the post-test of the present study, animals of TG did not exhibit a depressive state as they efficiently moved towards the central platform of the MWM, and did not manifest changes in the expression of movement, remaining at the borders of the maze without trying to escape from the water or assuming a flexed posture.

Changes in 5-HT release indirectly stimulate post-synaptic 5-HT receptors located in regions important for learning and memory and interfere with their functions. To prove this assumption, Du Jardin et al.[27] treated adult rats with para-chlorophenylalanine (pCPA), a compound that inhibits tryptophan hydroxylase and consequently reduces 5-HT synthesis.

The reduction in tryptophan resulted in serotonin depletion as demonstrated by changes in spatial memory (Y-maze) and object recognition tests.

Kenton, Boon and Cain[28] evaluated the individual and combined administration of pCPA, an inhibitor of 5-HT biosynthesis, and propranolol hydrochloride, a β-adrenergic antagonist, at doses of 500 mg/kg and 20 mg/kg per day, respectively, to 80 Long-Evans rats. The authors observed that the combination of pCPA and propranolol markedly impaired the performance of these animals in the visible platform and hidden platform versions of the MWM and in a sensorimotor test. However, little or no damage was observed when these substances were administered individually. The data revealed that the serotonergic and adrenergic systems influence adaptive and cognitive behavior and therefore play an important role in AD because of the diffuse nature of these systems and their capacity to act on different brain areas.

5-HT is synthesized by the biochemical transformation of the amino acid L-tryptophan. After conversion of L-tryptophan into 5-HT, the neurotransmitter is released into the synaptic cleft to bind to specific receptors, with serotonergic pathways projecting from the brainstem to all brain areas. In the present study, the serotoninergic pathway acted in the frontal cortex of the animals, a region that is essentially associated with attention, cognition and motor functions and that possesses innervation with the hippocampus, an area fundamental for memory and learning. The results showed that animals treated with the 5-HT precursor had improved concentration and cognition and treatment subsequently ameliorated the memory loss caused by dementia.

Piechal et al.[29] evaluated the effects of neonatal depletion of 5-HT on spatial memory and learning in the MWM. Sprague-Dawley rats were pretreated with desipramine, followed by intraventricular injection of a selective 5-HT neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT), which induces the depletion of 5-HT. After 3 months of treatment, the animals were tested in the MWM. An intense and permanent decrease in 5-HT levels was observed in the hippocampus and prefrontal and striatal region, but 5,7-DHT was unable to improve learning or spatial memory impairment.

The present study analyzed short-/medium-term memory and significant improvement in memory and learning was observed. Long-term memory was not evaluated, in contrast to the study of Piechal et al.[29] in which the animals were treated for 3 months and the selective 5-HT neurotoxin was ineffective. Thus, tryptophan was effective in the short term, but further studies are needed to evaluate its long-term effect.

Deficits in spatial memory are common in individuals with AD because of progressive damage to the temporal lobe. In the present study, using an experimental model of Alzheimer's, improvement in spatial memory was observed in TG and CG; however, animals of TG exhibited better behavioral performance as indicated by the greater reduction in mean displacement values pre- and post-induction of dementia.

Study limitations

Since the study was conducted at a public teaching institution, difficulties were encountered in the acquisition of materials for some parts of the work because of the lack of financial support. In addition, since this was an experimental study, part of the sample was lost, but was replaced to complete the study.

Conclusion

Treatment with L-tryptophan in an experimental model had beneficial effects on the reactive memory of treated animals compared to the group that did not receive any type of treatment.

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