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 Current Alzheimer Research, 2012, *9*, 1190-1198

Pretreatment with Memantine Prevents Alzheimer-Like Alterations Induced by Intrahippocampal Okadaic Acid Administration in Rats

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Abstract: Cerebral okadaic acid (OA) administration induces Alzheimer's disease (AD)-like phenotype in rats. Alterations in glutamate levels associated with hyperactivation of cyclin dependent kinase 5 (Cdk5) signaling pathway downstream Tau phosphorylation may participate in the genesis of this pathological phenotype. Here, we examined the efficacy of memantine (MN) pretreatment on reducing OA-induced AD-like phenotypes in rats. Wistar rats were given daily intraperitoneal injections of MN for 3 days and then given an intrahippocampal infusion of OA. Animals were divided into four groups: control (CO), MN, OA and MN/OA. Spontaneous locomotion and spatial memory performance were assessed by open field and Morris water maze respectively. Additionally, we measured glutamate levels in the cerebrospinal fluid (CSF) and the immunocontent of Cdk5, p35, p25 and phosphorylated Tau ($pTau^{Ser199/202}$) in the hippocampus. Spontaneous locomotion did not differ between groups. The OA group showed a significant decrease in spatial memory performance compared to all groups. The OA infusion also increased CSF glutamate levels and the immunocontents of Cdk5, p25 and $pTau^{Ser199/202}$ in the hippocampus. Conversely, pretreatment with MN prevented OA-induced spatial memory deficits and the increment of CSF glutamate level; which paralleled with normal immunocontents of Cdk5, p25 and $pTau^{Ser199/202}$ proteins. There were positive correlations between spatial memory performance and the neurochemical parameters. In summary, pretreatment with MN prevents spatial memory deficits induced by intrahippocampal OA administration in rats. The prevention of increase CSF glutamate levels, along with the reduced hippocampal phosphorylation of $Tau^{Ser199/202}$ by Cdk5/p25 signaling pathway, are the mechanisms proposed to participate in the prophylactic effects of MN in this AD-like model.

Keywords: Alzheimer´s disease, okadaic acid, memantine, glutamate, Cdk5, tau, learning and memory.

1. INTRODUCTION

Alzheimer's disease (AD) is an aging-associated neurodegenerative disease that causes important structural and neurochemical alterations and is associated with the progressive deterioration of cognitive function [1-3]. The neuropathological characterization of AD includes the accumulation of senile plaques (deposits of amyloid-β) and the aggregation of abnormal filaments of Tau protein into neurofibrillary tangles. These pathologies are present in brain regions involved in memory and cognition [4, 5].

For many years, acetyl cholinesterase inhibitors were the first choice in drugs for the treatment AD, but recently the glutamatergic system has also been shown to be a possible target for new drug therapies [6, 7]. Glutamate is the major excitatory neurotransmitter in the brain and plays fundamental roles in neurodevelopment, neuronal survival and in learning and memory processes through its interactions with ionotropic (AMPAr, KAr and NMDAr) and metabotropic receptors (mGluR) [8-10]. However, high amounts of glutamate in the synaptic cleft may cause receptor hyperactivation and neuronal death by excitotoxicity [11]. In this context, the N-methyl-D-aspartate receptor (NMDAr), a heterodimeric calcium ion channel, exerts a major role in a variety of neurodegenerative disorders, including AD [12, 13]. Excessive calcium influx through the ion channel activates the signaling pathways involved in neurodegeneration [10]. The cyclin dependent kinase (Cdk5) signaling pathway is physiologically regulated by p35 or p39 proteins. However, under conditions of high intracellular calcium concentrations, p35 is cleaved by calpain into p25. This cleavage allows the formation of the complex Cdk5/p25, which causes downstream aberrant phosphorylation of Tau. Indeed, several lines of evidence have associated the Cdk5 pathway with AD pathogenesis [14-16].

Memantine (MN), a non-competitive antagonist with a low affinity for the NR2B subunit of NMDAr, has been used in the treatment of AD to delay neurodegenerative processes and improve cognitive function [17-21]. The mechanism of MN action involves the blockade of an excessive influx of calcium through the NMDA receptor caused by glutamate hyper stimulation [22]. Recently, a pretreatment protocol with MN was shown to reverse neurochemical and behav-

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1567-2050/12 $58.00+.00

ioral alterations caused by an ischemic insult in rats [23]. Furthermore, when administered post-insult, MN improved the spatial memory deficit caused by a bilateral injection of okadaic acid (OA) [24]. These data provide support to the idea that MN could be used in prophylactic protocols to diminish the harmful effects of glutamate excitotoxicity in experimental models of neurological diseases.

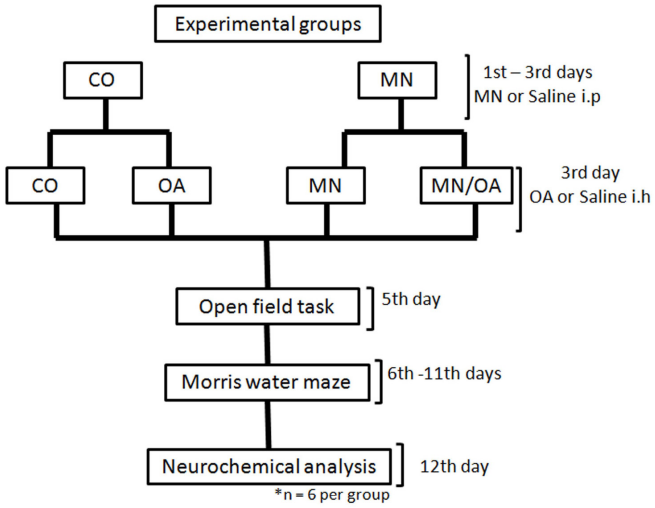

Fig. (1). Experimental Model. Animals received a daily intraperitoneal (i.p.) injection of MN (20 mg/kg) or saline (NaCl, 0.9%) over 3 consecutive days. On the third day, an intrahippocampal (i.h.) infusion of okadaic acid (100 ng) or saline (NaCl 0.9%) was made into the right hemisphere in the CA1. By the third day post-surgery, rats were considered suitable for behavioral experiments. After the *in vivo* experiments we choose randomly 6 animals per group for the neurochemical assays.

The intracerebral administration of OA causes a selective inhibition of the serine/threonine phosphatase 1(PP1) and 2A (PP2A) and exacerbates kinase activities [25]. This imbalance in phosphorylation/ dephosphorylation status induces AD-like phenotype including cognitive deficits, and hyperphosphorylation of NMDAr receptor subunits and Tau protein [16, 26-28]. Tau is a major microtubule-associated protein whose hyperphosphorylated form is considered one of the hallmark alterations reported in neurons of Alzheimer's disease patients. The increase in Tau phosphorylation is believed to cause neuronal death by destabilization of the cytoskeleton, disruption of axonal transport, microglial activation, mitochondrial dysfunction and increased generation of reactive oxygen species [24, 29-31].

This study aimed to investigate whether the prophylactic use of MN is capable of preventing AD-like phenotype induced by OA in rats. Our data demonstrate that MN prevented spatial memory deficits through the modulation of brain glutamate levels and phosphorylation of Tau by Cdk5/p25 signaling pathway.

2. MATERIAL AND METHODS

2.1. Animals

Male Wistar rats (400-500 g), 4-5 months old, were obtained from the State Foundation for Health Science Research (FEPPS, Porto Alegre/RS, Brazil). Animals were placed into a controlled temperature room (22 ºC) under a 12 h light/12 h dark cycle (lights on at 7 am) and had free access to food and water. A total of fifty-nine (n=59) rats were divided into four groups: control (CO, n=17), memantine (MN,n=14), okadaic acid (OA,n=14) and memantine/okadaic acid (MN/OA, n=14). To avoid social isolation, we kept 4 animals per cage [32]. All behavioral tests were performed in normal cycle between 9:00 a.m. and 5:00 p.m. All experiments were in agreement with the Committee on the Care and Use of Experimental Animal Resources, UFRGS, Brazil.

2.2. Drugs

Memantine (Ref. M-9292, Sigma, USA) and okadaic acid (Ref. O8010, Sigma, USA) were dissolved in saline (NaCl, 0.9%) at a concentration of 20mg/ml and 50ng/µL, respectively.

2.3. Treatment and Surgical Procedure

Animals received a daily intraperitoneal (i.p.) injection of memantine (20 mg/kg) or saline (NaCl, 0.9%) over 3 consecutive days. The dose of MN was based on work where MN was shown to prevent neural damage caused by focal ischemia in rats [23]. In addition, Abdel-Aal *et al.* 2011 reported that sixty days of daily MN (20 mg/kg) administration in rats prevented aluminum-induced cognitive deficits and did not cause alterations in motor integrity and coordination [33]. On the third day of MN injections, animals were anesthetized by an i.p. injection of ketamine (Cetamin, Schering-Plough Coopers, Brazil, 100 mg/kg body weight) and xylazine (Coopazine, Syntec, Brazil, 10 mg/kg body weight). An intrahippocampal (i.h.) infusion of 2uL okadaic acid (100 ng) or saline (NaCl 0.9%) was made into the right hemisphere in the CA1 region at the following coordinate location, with reference to bregma: A -3.6, L 2.0, and V 2.4 [34, 35]. This dose of OA was reported to cause spatial learning impairment and hippocampal neurodegeneration in rats [36]. Furthermore, the same dose also caused pyramidal cell loss in the CA1 neurons by apoptosis, similar to those observed in neurodegenerative diseases, like AD [37]. By the third day post-surgery, rats showed normal food intake, water consumption and spontaneous locomotion in the cage and were considered suitable for *in vivo* experiments.

2.4. Open Field Task

The open field test is commonly used to evaluate spontaneous locomotor activity. The apparatus for this test was a circular black box with a 60 cm diameter and a 50 cm height. The experiments were conducted in a sound-attenuated room under low-intensity light (12 lx). The rats (n=10 per group) were placed in the center of the arena and spontaneous locomotion, exploratory activity and anxiety-like behavior was recorded with a video camera for 10 min. In order to analyze anxiety-like behavior, we created a virtual central circular zone (30 cm of diameter) using the analysis software (for review, see Prut *et al.* 2003 [38]). Animals that spent less time in central zone were considered to display anxiety-like behavior. All analyses were performed using a computer-operated tracking system (Any-maze, Stoelting, Woods Dale, IL).

2.5. Morris Water Maze Task

To evaluate spatial memory, we conducted the Morris water maze task (MWM), as described by Muller *et al.* 2010 [39]. The apparatus was a black circular pool with a 190 cm diameter and 70 cm height and the water temperature was maintained at 21 ± 1 °C. Rats (CO: n= 17, MN n=14, OA: n=14 and MN/OA: n=14) were trained daily in a 4-trial water maze task up to 4 consecutive days. Each trial lasted up to 60 s, including 20 s of rest on a hidden black platform. During training, the animals learned to escape from the water by finding a hidden black platform submerged about 2 cm below the water surface in a fixed location. If an animal failed to find the platform in 60 s, it was removed from the water, gently placed on the platform and allowed to rest for 20 s. Rats were returned to their home cages immediately after each daily training session. The maze was located in a well-lit white room with several visual stimuli hanging on the walls to provide spatial cues. Escape latency was defined as the time to find the platform during each trial and was used as an indicator of learning. A probe test without the platform was performed on the fifth day, and the time spent in the target quadrant was used as an indicator of memory retention.

2.6. Cerebrospinal Fluid (CSF) Sampling

On the 11th day, after the last MWM session, rats (n=6 per group) were anesthetized with ketamine (Cetamin, Schering-Plough Coopers, Brazil, 100 mg/kg body weight) and xylazine (Coopazine, Syntec, Brazil, 10 mg/kg body weight) and placed in a stereotaxic apparatus. The CSF was collected (40 to 80 µL) by direct puncture of the cisterna magna with an insulin syringe (27 gauge × 1/2-inch length) and stored at - 80 °C. Samples with blood contamination were discarded.

2.7. High-Performance Liquid Chromatography (HPLC) Procedure

HPLC was performed to measure glutamate levels in the aliquots obtained from the CSF cell-free supernatants. The measurement was performed as previously described (Schmidt *et al.*, 2009). Analyses were performed with the Shimadzu Class-VP chromatography system, which consisted of a quaternary gradient pump with vacuum degassing and piston desalting modules, a Shimadzu SIL-10AF auto-injector valve with a 50 mL loop and a UV detector (Shimadzu, Kyoto, Japan). Separations were achieved on a Supelco 250 mm × 4.6 mm, 5 µm particle size column (Supelco, St Louis, MO, USA). The mobile phase flowed at a rate of 1.2 mL/min, and the column temperature was 24 °C. The buffer composition remained unchanged (Buffer A: 150 mmol/L phosphate buffer, pH 6.0, containing 150 mmol/L potassium chloride; Buffer B: 15% acetonitrile in Buffer A). The gradient profile was modified to the following content of Buffer B in the mobile phase: 0% at 0.00 min, 2% at 0.05 min, 7% at 2.45 min, 50% at 10.00 min, 100% at 11.00 min, and 0% at 12.40 min. Samples of 10 µl were injected into the injection valve loop. Absorbance was read at 360 and 455 nm (emission and excitation, respectively). CSF glutamate levels are expressed as the mean ± SEM in micromoles.

2.8. Western Blotting

For Western blot analysis, we used ipsilateral hippocampus (n=6 per group). Hippocampal homogenates were prepared in PIK buffer (1 % NP-40, 150 mM NaCl, 20 mM Tris, pH 7.4, 10 % glycerol, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 400 µM sodium vanadate, 0.2 mM PMSF, 1 µg/ml leupeptin, 1 µg/ml aprotinin, and 0.1 % phosphatase inhibitor cocktails I and II from Sigma-Aldrich) and centrifuged. Supernatants were collected and the total protein was measured using Peterson's method [40]. Samples containing 40 µg of protein from the hippocampal homogenate were separated by electrophoresis on a polyacrylamide gel and electrotransferred to PVDF membranes. Protein bands within each sample lane were compared to standard molecular weight markers (Precision Plus Protein™ Dual Color Standards #161-0374), which were used to identify the molecular weight of protein of interest. We performed at least 3 replicate gels for each sample of the same animals. Non-specific binding sites were blocked with Tween–Tris buffered saline (TTBS, 100 mM Tris–HCl, pH 7.5) with 5% albumin for 2 h. Samples were incubated overnight at 4 ^{o}C with monoclonal and polyclonal primary antibodies against Cdk5 (Cell Signaling Technology, 1:1000), p25/35 (Cell Signaling Technology) pTau$^{ser199/202}$ (Invitrogen, 1:1000), Tau (Santa Cruz, 1:500) and actin (Sigma, 1:5000). Following primary antibody incubation, the membranes were incubated with secondary antibodies (anti-rabbit, Cell Signaling Technology, 1:3000; anti-mouse, Santa Cruz Technology, 1:5000) for 2 h at room temperature. The films were scanned and the band intensity was analyzed using ImageJ software [41].

2.9. Statistical Analysis

Results are presented as the means ± SEM. The data from the water maze task were analyzed with a repeated-measures analysis of variance (ANOVA), followed by Tukey's post-hoc test. Differences between all groups were analyzed with ANOVA with a Tukey's post-hoc test. Correlations between the measures of cognitive function and protein levels were analyzed by Pearson's correlation. Differences were considered statistically significant if $p<0.05$.

3. RESULTS

3.1. Effects of MN and OA On Locomotor and Exploratory Activity

To analyze the effects of MN and OA in locomotor and exploratory activity, we performed the open field task. The administration of MN and/or OA did not cause significant changes across time in the locomotor and exploratory activity in the open field task Fig. (**2A**), ($F_{[1,36\ ]} = 0.632$, $p = 0.432$). In addition, neither distance traveled Fig. (**2B**), ($F_{[3,36]}=1.418$, $p=0.2534$) nor mean speed Fig. (**2C**), ($F_{[3,36]}=1.491$, $p=0.2335$) was affected, suggesting that the treatment did not cause locomotor deficit. Furthermore, there were no statistical differences among groups in either the time spent ($F_{[3,36]}=1.917$, $p=0.1442$) or the total distance travelled in the central zone ($F_{[3,36]=}1.634$, $p= 0.1987$) suggesting no signs of anxiety-like behavior (data not shown).

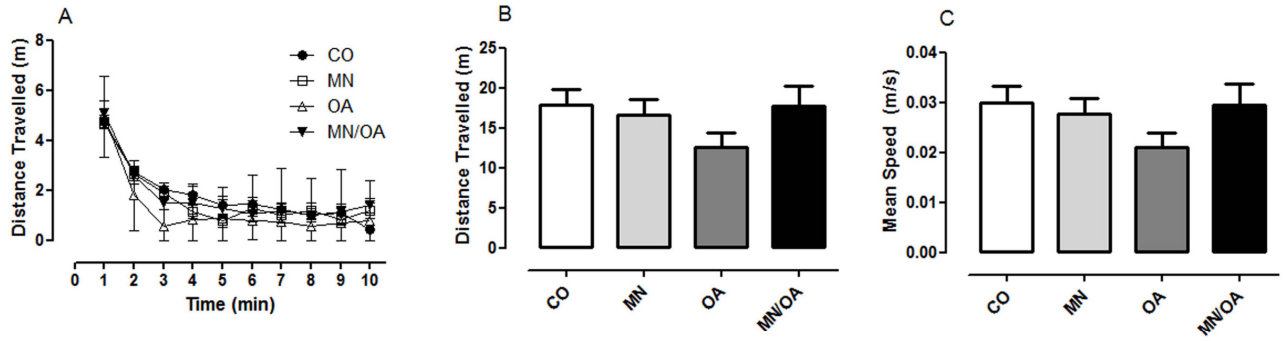

Fig. (2). Open Field Task. The spontaneous locomotor and exploratory activities were not affected by OA and MN. (**A**) Distance traveled per minute, showing exploratory and locomotor activity. (**B**) Total distance travelled. (**C**) Mean speed. Groups: control (CO), memantine (MN), okadaic acid (OA) and okadaic acid/memantine (MN/OA); n=10 per group. Data are represented as the mean ± SEM. *p<0.05 between groups.

3.2. Effects of MN and OA In Spatial Memory

To analyze the effects of MN and OA on spatial memory, we subjected the rats to the Morris water Maze Task (MWM). During the acquisition sessions (4 days), all groups showed improvements in their performances across days and took less time to find the hidden platform Fig. (**3A**), ($F_{[3,165]}$= 47.058, p=0.0001). On the fourth day, the OA group reached a performance plateau and took more time to find the platform compared to other groups Fig. (**3A**), ($F_{[3,55]}$= 4,191, p= 0.0096). Further, MN prevented the impairment caused by OA on the fourth day Fig. (**3A**), ($F_{[1,55]}$ = 4.428, p=0.040). In the retention session (day 5), the OA group showed impaired spatial memory performance compared to the other groups Fig. (**3B**), ($F_{[3,55]}$= 5.161, p=0.0032). There were no statistical differences among groups regarding total distance traveled Fig. (**3C**), ($F_{[3,55]}$= 0.6523, p=0.5849) or mean speed Fig. (**3D**), ($F_{[3,55]}$= 0.7080, p=0.5514).

3.3. Effects of MN and OA Administration on Hippocampal Cdk5 Signaling, Tau Phosphorylation (Ser199/202) and Glutamate CSF Levels

The i.h. infusion of OA increased the hippocampal immunocontent of Cdk5 and p25/p35 ratio; however, three days of pretreatment with MN prevented these increases Fig. (**4A**) ($F_{[3,20]}$=6.061, p= 0.0042) Fig. (**4B**) and ($F_{[3,20]}$=3.764, p=0.0272). Further, OA administration significantly enhanced CSF glutamate levels, which was prevented by MN Fig. (**4C**) ($F_{[3,20]}$= 10.88, p=0.0002). Finally, intrahippocampal OA infusion caused an increase in the immunocontent of $pTau^{Ser199/202}$ but pretreatment with MN prevented this augmentation Fig. (**4D**) ($F_{[3,20]}$= 4.769 , p= 0.0115).

3.4. Correlation Between MWM and Neurochemical Assays

There were positive correlations between the latency to find the platform on day 4 of MWM and the immunocontent of Cdk5 Fig. (**5A**, R=0.6224, p=0.0012), ratio p25/p35 Fig. (**5B**, R=0.5624, p=0.0042) and $pTau^{Ser199/202}$ Fig. (**5C**, R=0.4126, p=0.0451). Moreover, CSF glutamate level was strongly correlated with increased latency on day 4 Fig. (**5**, R= 0.7302, p=0.0001).

4. DISCUSSION

Alzheimer's disease is multifactorial and heterogeneous, and thus offers multiple therapeutic opportunities [42]. The present study showed the potential of MN pretreatment as a strategy for preventing AD-like alterations induced by intrahippocampal OA infusion in rats. Indeed, MN prophylaxis prevented spatial memory impairment on Morris water maze task (MWM), which paralleled decreased expression of Cdk5, p25 and $pTau^{Ser199/202}$, and CSF glutamate levels.

Inhibition of PP2A activity by OA seems to have no effect on locomotor speed, motor coordination or sensorimotor ability [37, 43]. In fact, our results showed no significant statistical differences between groups in the spontaneous locomotion, mean speed and exploratory activities in the open field task. Although the data appear to indicate a slower speed in the open field in the OA group, the mean speed did not reach statistical significance (p=0.2335).The lack of significant locomotor deficits in the open field task permits further assessment in MWM. Zhang and Simpkins (2010) have already reported impaired performance in the Morris water maze task after an intra hippocampal OA administration. Similarly, we showed impaired spatial memory performance on MWM after intra hippocampal OA infusion. Although rats treated with MN appear to show a decreased time spent in the target quadrant, this observation is not supported by the statistical analysis. Of note, rats treated with MN after a bilateral intracerebroventricular infusion of OA showed improved performance on MWM [24].

By using a prophylactic approach we showed that MN prevented the course of molecular alterations involved in the development of memory deficits induced by OA. It has been suggested that protein phosphatase (PP1 and PP2A) activity plays an important role in normal brain physiology by controlling the neuronal dephosphorylation system, which, when dysfunctional, results in the hyperphosphorylation of Tau protein and memory deficits [44]. Although a normal PP1 activity is implicated in the improvement of learning and memory processes [45], it does not account for the majority of phosphatase activity inhibited by OA. The inhibition of PP2A by OA is achieved at concentrations up to 100 times lower than those required to inhibit PP1[46]. Considering the dose used in this work, we believe that OA is preferentially

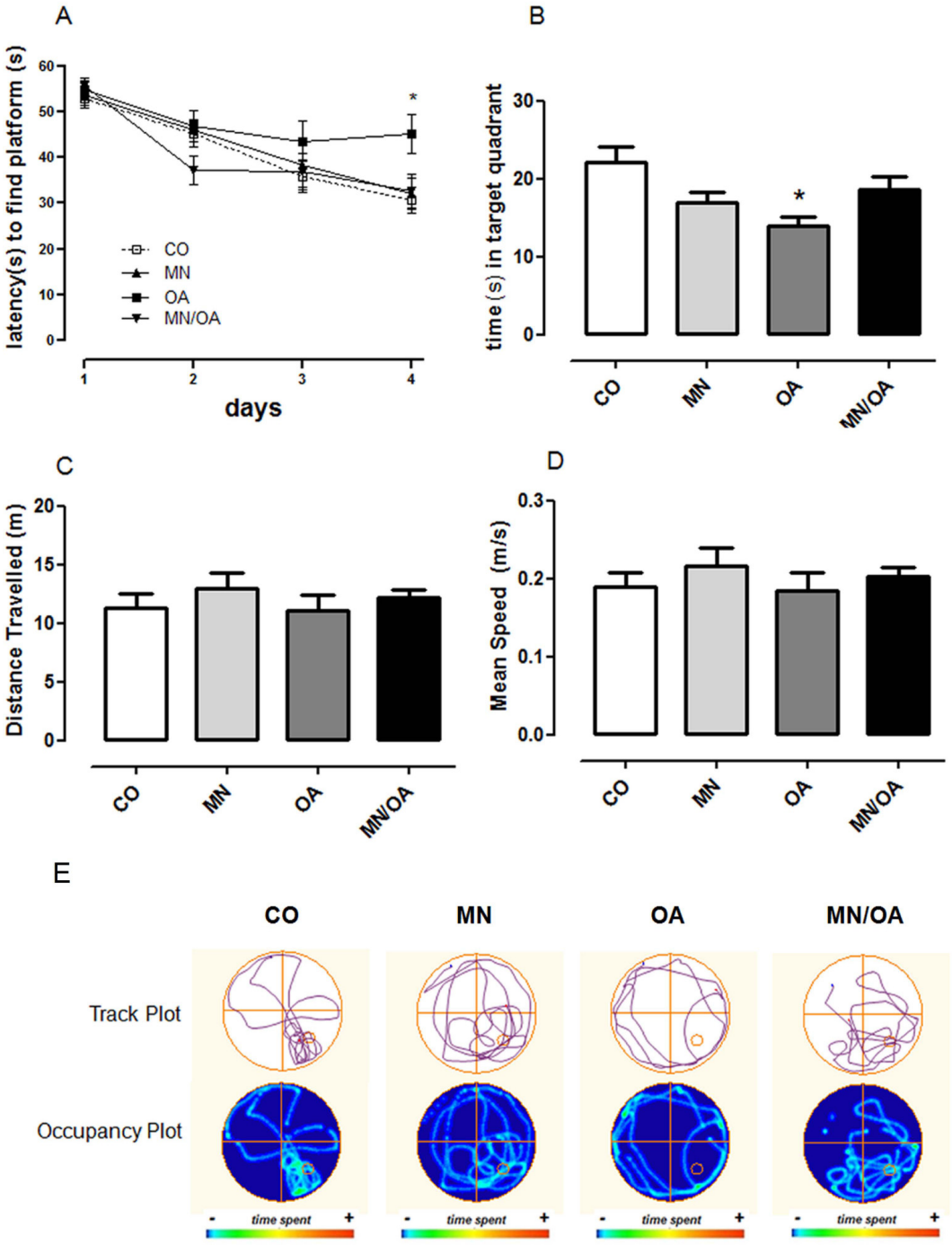

Fig. (3). Morris water maze task. OA impaired both the acquisition and retention memory performances and MN prevented these deficits. (**A**) Acquisition task: the latency to find the platform was used to assess learning ability. (**B**) Retention task: the time in the target quadrant was used to assess memory retention. (**C**) Total distance travelled. (**D**) Mean speed. (**E**) Representative track and occupancy plots obtained by video-tracking software (ANY-mazeH, Stoelting CO, USA), during the retention task. Groups: control (CO, n= 17), memantine (MN, n = 14), okadaic acid (OA, n = 14) and okadaic acid/memantine (MN/OA, n = 14). Data are represented as the mean ± SEM. *p<0.05 between groups.

inhibiting PP2A activity. Interestingly, it was demonstrated that the inhibition of PP2A by a hippocampal infusion of OA caused a transient impairment of the spatial memory performance and a persistent neurodegeneration [36, 37]. In contrast, we detected persistent spatial memory deficits that affected distinct phases of the MWM task (acquisition and retention).

One of the remarkable characteristics of AD is the progressive neurodegeneration associated with cognitive decline [1-3]. The mechanisms underlying brain degeneration and

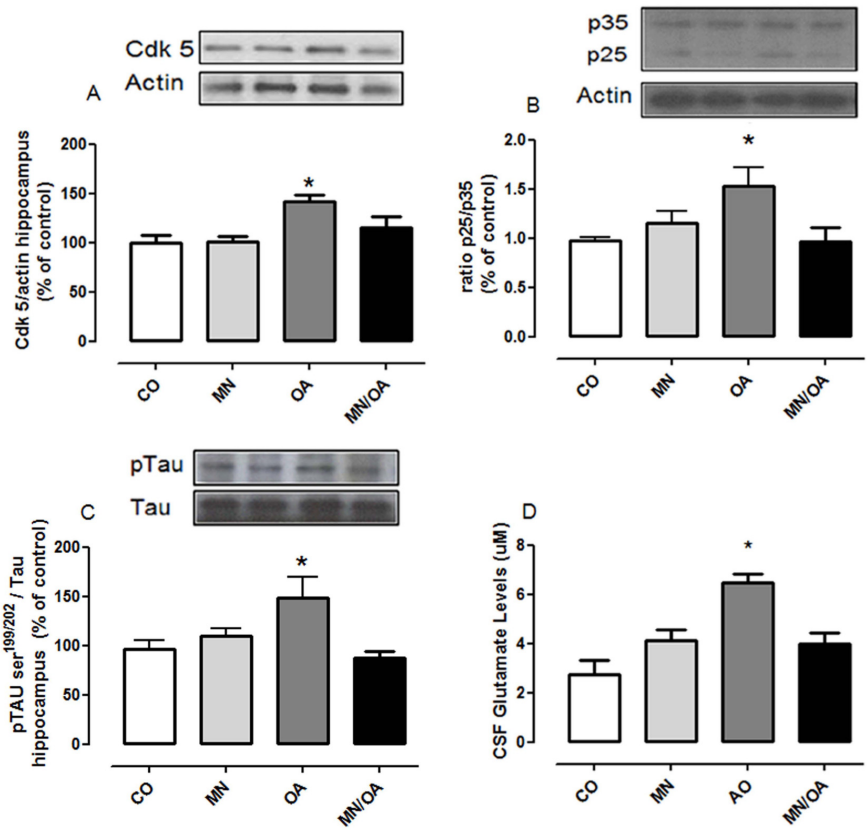

Fig. (4). MN prevent neurochemical alterations (Cdk5, p25, pTau$^{Ser199/202}$, glutamate) induced by i.h. OA administration. Western blots of proteins involved in Cdk5 signaling pathway obtained from hippocampal homogenates: (**A**) immunocontent of Cdk5, (**B**) ratio p25/p35 (**C**) Phosphorylation state of Tau$^{Ser199/202}$. (**D**) Glutamate levels in cerebrospinal fluid. Groups: control (CO), memantine (MN), okadaic acid (OA) and okadaic acid/memantine (MN/OA); n=6 per group. Data are represented as the mean ± SEM. *p<0.05 between groups.

memory deficits in AD include the hyperactivation of the glutamatergic system caused by high levels of glutamate in the synaptic cleft [47]. The infusion of OA, in turn, disrupts the balance between phosphorylation and dephosphorylation, such that there is a disproportionate level of regulatory proteins in a phosphorylated state [28, 44], which negatively affects neuronal function and activates neurodegenerative processes probably due to Tau hyperphosphorylation (Sun *et al.*, 2003). In this sense, MN therapy has been addressed to inhibit Tau hyperphosphorylation, neuronal death, and to prevent the inhibition of PP2A activity [48].

Moreover, among the putative targets of OA neurotoxicity is the persistent activation of NMDA glutamate receptor caused by increased glutamate levels. We can speculate that under these circumstances, there is an increased calcium influx through the ion channel of the NMDA receptor. Indeed, we showed that OA increases CSF glutamate levels suggesting a mechanism that mimics glutamate excitotoxicity. Similarly, glutamate levels have been found to be significantly elevated in the CSF of AD patients [49]. On the other hand, three days of MN administration prevented the increases in CSF glutamate levels caused by OA, thus suggesting that MN modulates the neuronal/glial mechanisms involved in synthesis, release and uptake of glutamate. Accordingly, it has been suggested that the neuroprotective effect of MN involves the depression of glutamate release by neurons specially when excessive glutamate release occurs [50]. We also demonstrated that MN prevented the aberrant expression of pTau$^{Ser199/202}$ induced by OA. Interestingly, these sites are downstream phosphorylated by abnormal Cdk5 activity [51, 52].

We also observed increases in hippocampal expression of Cdk5 and its pathological activator p25 after OA injection. As stated above, these increments were accompanied by an increased phosphorylation status of Tau$^{Ser199/202}$. In contrast, MN pretreatment prevented the increase expression of Cdk5, p25 and pTau$^{Ser199/202}$ in the hippocampus. Considerable evidence suggests that Cdk5 is required for the proper development of the mammalian central nervous system [14] but may also be implicated in neuronal death in neurodegenerative disorders [53, 54]. For instance, Cdk5 physiologically modulates NMDA activity by the phosphorylation of NR2A and NR2B subunits [16, 47, 55], however the pathological activation of NMDAr by Cdk5/p25 causes excessive calcium influx into neurons and functional deficits/death [56]. In support of these data, we showed that the immunocontent of Cdk5 and p25/p35 in hippocampus positively correlates with memory deficits on day 4 of the MWM. In fact, many studies demonstrate the importance of Cdk5 signaling pathway and its effectors in the learning and memory function and suggest this protein as a novel therapeutic target in neurodegenerative diseases [57]. Similarly, CSF glutamate levels and immunocontent of pTau$^{Ser199/202}$ were correlated with spatial

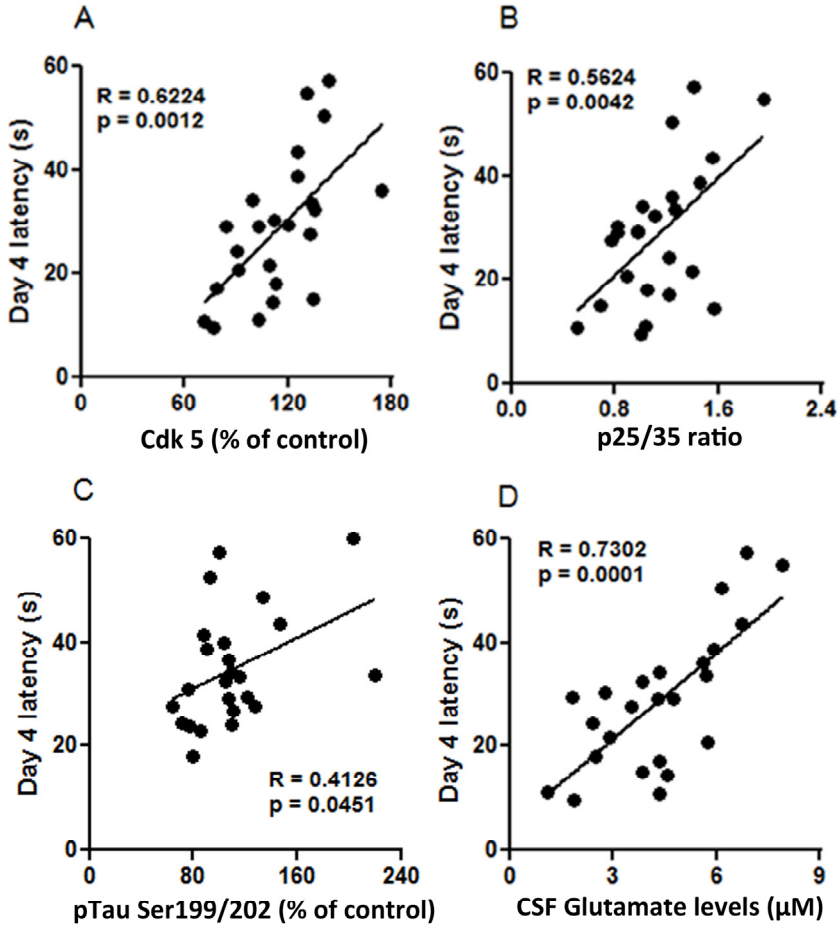

Fig. (5). Correlation analysis between memory performance on MWM task and neurochemical assays: **A**) Latency to find the platform on day 4 and Cdk5 levels in hippocampus. **B**) Latency to find the platform on day 4 and p25/p35 ratio in hippocampus. **C**) Latency to find the platform on day 4 and pTau$^{Ser199/202}$ levels in hippocampus. **D**) Latency to find the platform on day 4 glutamate levels in cerebrospinal fluid; n = 24. Data represented by one animal per point.* p< 0.05.

munocontent of pTau$^{Ser199/202}$ were correlated with spatial memory deficits, reinforcing the idea that these elements are active participants in the pathological processes that culminate in AD. Interestingly, some clinical studies already showed positive correlations between cognitive deficits with Tau and glutamate CSF levels [58-60].

The prophylactic use of MN is not typical in clinical practice; however, experimental evidence supports the notion that the molecular and cellular mechanisms involved in etiology of neuropathological changes and cognitive deficits can be targeted by a prophylactic regimen before the onset of dementia symptoms. In this context, there is increasing interest in the search for new tools, methods and molecular markers to enable an early diagnosis of AD or to detect, prior to symptom onset, individuals who are at risk of developing AD [61-66]. An early diagnosis coupled with a pretreatment could provide opportunities to prevent and/or delay the behavioral, neurochemical and neuroanatomical alterations associated with neurodegenerative disorders.

5. CONCLUSION

In summary, pretreatment with MN prevents spatial memory deficits induced by intrahippocampal OA administration in rats. The prevention of increase CSF glutamate levels, along with the reduced hippocampal phosphorylation of Tau$^{Ser199/202}$ by Cdk5/p25 signaling pathway, are the mechanisms proposed to participate in the prophylactic effects of MN in this AD-like model.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

This work was supported by the following Brazilian agencies and grants: CNPq, CAPES, FAPERGS, Brazilian Institute of Neuroscience - IBNnet FINEP, INCT - Excitotoxicity and Neuroprotection.

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Received: ????????????? **Revised:** ????????????? **Accepted:** ?????????????