EFFECTS OF RIVASTIGMINE ON LEARNING AND MEMORY PROCESSES IN RATS - ACTIVE AVOIDANCE TEST

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ABSTRACT

Rivastigmine belongs to the second generation cholinesterase inhibitors using for treatment of Alzheimer's disease. In scientific literature there are data that rivastigmine improves cognitive functions in experimental animals and humans.

The aim of our study was to compare the effects of two doses rivastigmine on learning and memory processes in rats using active avoidance test. The male Wistar rats (9 per group, with body weight 220-240 g) was treated with: 1st - Saline 0.1ml/100g body weight (controls) p.o.; 2nd - Rivastigmine 1 mg/kg p.o.; 3rd - Rivastigmine 2 mg/kg p.o. All groups of animals were trained in shuttle-box active avoidance test, using original made standard apparatus (Ugo Basile, Italy) with training parameters for rats. In active avoidance test learning session was performed 5 consecutive days and consist 30 trails, memory retention was done 7 days later. The following behavioral parameters were observed: number of correct responses (avoidances), number of escapes from food shocks and number of intertrial crossings. The comparison between groups made by Instat computer program using analysis of variance (ANOVA for repeated measurements).

In active avoidance test control group significantly increased the number of avoidances on 4th and 5th day learning and on memory retention test in comparison with 1st day. The rats with Rivastigmine 1 mg/kg statistically significant increased the number of correct responses on all days learning session, but not kept it in memory test, compared to the same day control group. The animals treated with Rivastigmine 2 mg/kg made the number of avoidances similar to the control group on learning and memory tests. Control rats did not change the number of escape on learning and memory sessions. The rats with Rivastigmine 1 mg/kg increased the number of avoidances on the 4th day learning. The animals with Rivastigmine 2 mg/kg significantly increased the number of escapes on 2nd and 5th day learning and on memory retention test. The two groups with Rivastigmine increased the number of intertrial crossings on 2nd, 3rd, 4th and 5th days learning session. On memoty test only rodents with higher dose Rivastigmine (2 mg/kg) significantly increased the number of intertrial crossings.

Our results allow us to conclude that Rivastigmine improves better learning than memory processes and this effect is not depend from doses applied. Both doses have similar stimulating effect on moving activity in rats.

Key words: *Rivastigmine, shuttle-box, learning, memory, rats*

INTRODUCTION

Alzheimer's disease (AD), the most common cause of dementia is characterized clinically by ongoing declines in cognitive and functional ability and the emergence of behavioral and psychological symptoms (Blennow et al., 2006). Presently, available symptomatic therapies for treatment of AD have been based on known neurotransmitter dysfunctions associated with the illness. The second generation cholinesterase inhibitors (donepezil, rivastigmine, galantamine) and N-methyl D-aspartate receptor antagonist memantine have been widely prescribed and studied (Lanctôt et al., 2009). The rationale basis for the use of cholinesterase inhibitors in the treatment of AD is to correct the cholinergic hypofunction by preventing acethylcholine hydrolysis in the central nervous system. This leads to an increase in extracellular levels of acetylcholine released from the residual cholinergic neurons, which in turn may restore cholinergic neurotransmission (Scali et al., 2002).

Rivasrigmine is a carbamate-based, reversible, noncompetitive inhibitor of both acethylcholinesterase and butyrylcholinesterase. Its inhibitory effect is of long duration and is relatively specific to the central nervous system. Rivastigmine is approved for treatment of mild-to-moderate Alzheimer's disease and for mild-to-moderate dementia related to Parkinson's disease (Yuede et al., 2007).

The **AIM** of our study was to compare the effects of two doses rivastigmine on learning and memory processes in rats using active avoidance test.

MATERIAL AND METHOD

All experiments were carried out according to the guidelines for the use of laboratory animals in EU and Bulgaria. Official permission for the study was obtained by Bulgarian Food Safety Agency №49/30.06.2011 and Ethics Committee of the Medical University Plovdiv №3/05.07.2012.

Drug

Rivasrigmine (S)-N-ethyl-3-[(1-dimethyl-amino)ethyl]-N-methyl-phenylcarbamate hydrogentartrate (Novartis Pharma) was used in this study.

Animals

Male Wistar rats weighting 220-240 g were divided into 3 groups of 9. Rats were kept under standard laboratory conditions in a 08:00-20:00 h light/dark cycle and were provided with food and water *ad libitum*. The drug was administered per orally 60 minutes before testing. The following experimental groups were used: A: saline (0.1 ml/100 g body weight); B: Rivastigmine 1.0 mg/kg and C: Rivastigmine 2.0 mg/kg.

Behavioral test

The active avoidance test with negative reinforcement was performed in a shuttle box. A conventional shuttle-box was used, originally made as an automatic reflex conditioner (Ugo Basile, Italy). Learning sessions were held for 5 days and consisted of 30 trials (6 sec light and buzzer, 670 Hz and 70 dB, followed within 3 sec by random 0.4 mA foot electrical stimulation and 12 sec pause). Seven days later a 1-day memory retention test was oerformed using the same parameters without foot stimulation.

The following behavioral sings were observed: i) number of correct responses on conditioned stimuli, i.e. avoidances; ii) number of escapes from foot stimulation (unconditioned stimuli responses); iii) number of intertrial crossings.

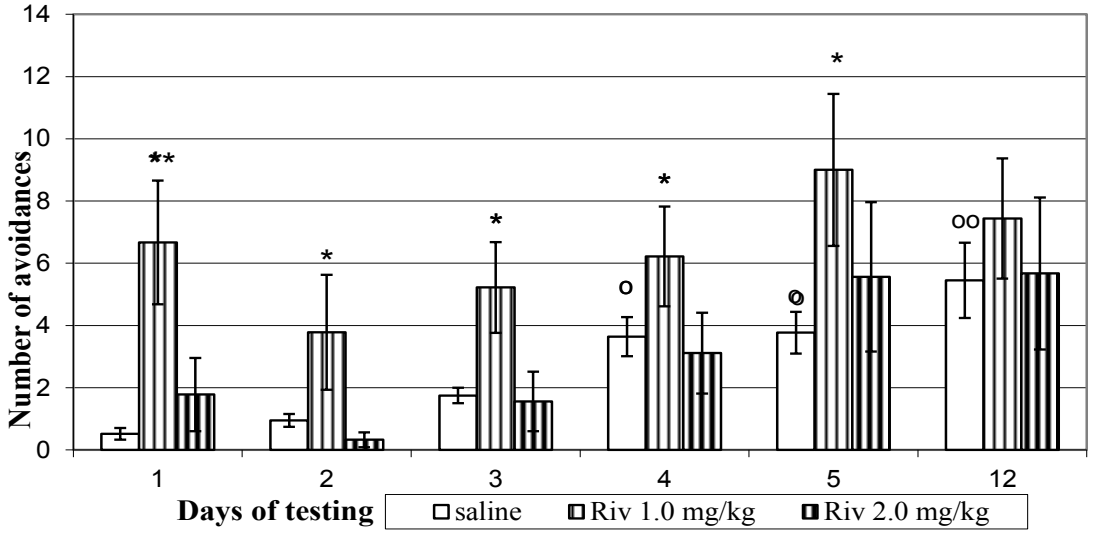
Statistical evaluation

The means ± SEM for each group of rats were calculated using Instat computer program. A two-way ANOVA for repeated measurements was used to compare different groups with the respective controls with the Turkey-Kramer multiple comparison test.

RESULTS

In active avoidance test, the control rats showed a significantly increased number of conditioned stimuli responses, i.e., avoidances on 4^{th} ($p<0.05$) and 5^{th} ($p<0.01$) days learning and on memory retention test ($p<0.01$) in comparison with 1^{st} day. The rats with Rivastigmine 1.0 mg/kg statistically significant increased the number of correct responses on all days learning session: 1^{st} day ($p<0.01$), 2^{nd}, 3^{rd}, 4^{th} and 5^{th} days ($p<0.05$), but do not kept it in memory retention test, compared to the same day control group. The animals treated with Rivastigmine 2.0 mg/kg showed the number of avoidances similar to the control group on learning and memory tests (Figure 1).

Figure 1. Effects of rivastigmine on learning and memory proceses in rats. Shuttle-box active avoidance test

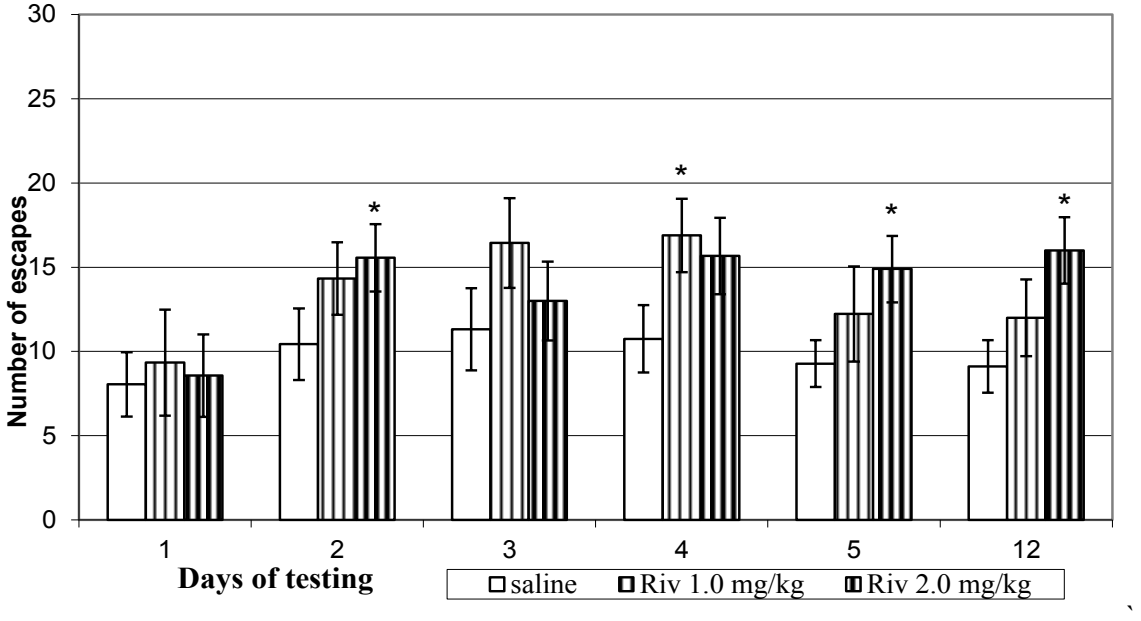


^{0} p<0,05 and ^{00} p<0,01 compared to the 1st day control;
* p<0,05 and ** p<0,01 compared to the same day control;

Control rats did not change the number of escapes on learning and memory sessions.

The rats with Rivastigmine 1.0 mg/kg increased the number of avoidances on the 4th day learning (p<0.05), compared to the same day saline group. The animals with Rivastigmine 2.0 mg/kg significantly increased the number of escapes on 2nd and 5th day learning (p<0.05) and on memory retention test (p<0.05), in comparison with the same day control group (Figure 2).

Figure 2. Effects of rivastigmine on learning and memory proceses in rats. Shuttle-box active avoidance test

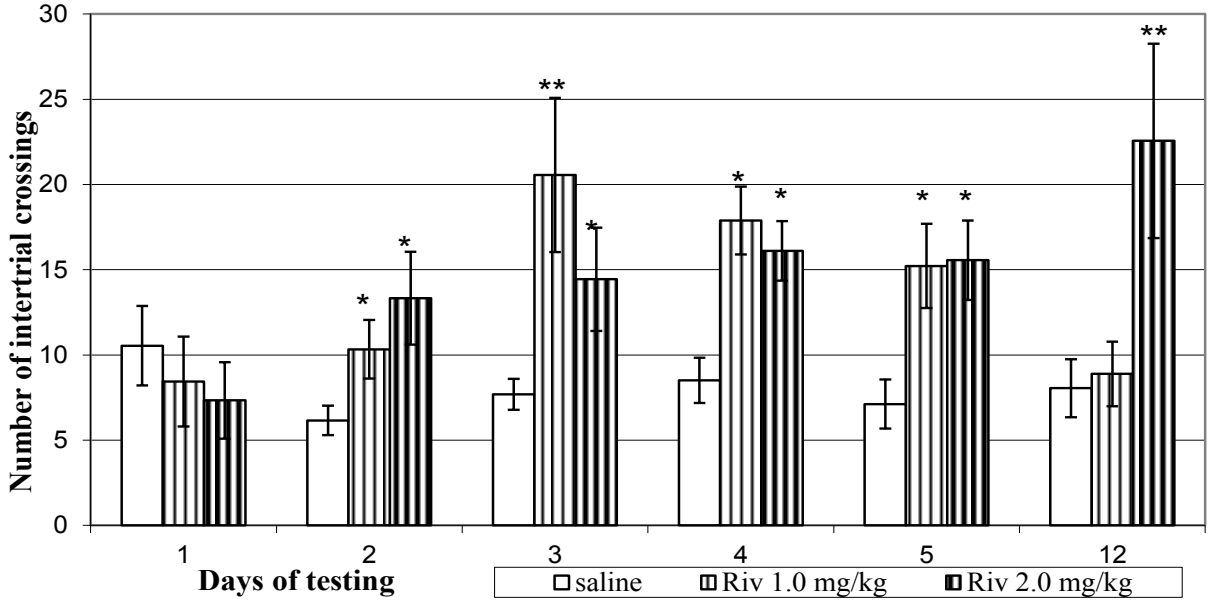


* p<0,05 compared to the same day control;

There were no clear differences between the number of intertrial crossings made by control rats during the 5-days learning sessions and memory test.

The rats with Rivastigmine 1.0 mg/kg significantly increased the number of intertrial crossings on 2nd day ($p<0.05$), 3rd ($p<0.01$), 4th and 5th days ($p<0.05$) learning, compared to the same day control group. In memory retention test this group made intertrial crossings similar to the saline group. The group with Rivastigmine 2.0 mg/kg significantly increased the number of intertrial crossings on 2nd, 3rd, 4th and 5th days learning session ($p<0.05$) and on memory test ($p<0.01$) compared to the same day controls (Figure 3).

Figure 3. Effects of rivastigmine on learning and memory proceses in rats. Shuttle-box active avoidance test



* $p<0,05$ and ** $p<0,01$ compared to the same day control;

DISCUSSION

Results from the active avoidance test showed that control rats learned the task and results from the memory retention test showed that they had retained the knowledge. The animals received smaller dose Rivastigmine learned better on all 5-days learning, but not made long term memory traces. These data suggest that the effects of Rivastigmine on learning and memory processes have not dose-response relationship.

The observation tendency towards an increase in the number of unconditioned stimuli in rats treated with rivastigmine showed the facilitation of learning in animals treated with cholinesterase inhibitors.

Rivastigmine in two applied of us doses increased locomotor activity (number of intertrial crossings) in experimental animals. Executive increasing locomotor activity have been observed in memory retention test only in rats with higher dose rivastigmine.

Because cholinergic function is required for short-term memory, it was determined that cholinergic deficit in AD was also responsible for the short-term memory deficit. Acetylcholinesterase inhibitors can augment levels of acetylcholine in the brain to compensate the loss of cholinergic function (Akhondzadeh and Abbasy, 2006). Scali et al., (2002) find that the linear relationship exists between the cholinesterase inhibition and extracellular acethylcholine levels in the cerebral cortex and hippocampus in rats, treated 21 days with rivastigmine. In the brain cortex rivastigmine led to 52% cholinesterase inhibition and 6 fold acethylcholine increase. Age-

associated changes in the function of muscarinic autoreceptors have been reported (Vannucchi et al., 1997) as well. According to Tanaka et al. (1994) repeated rivastigmine administration prevented the age-associated decrease in muscarinic receptors (M_1) binding sites.

Other scientists (Abdel-Aal et al., 2011) studied effects of rivastigmine at doses similar of ours 0.5, 1.0, 1.5 and 2.5 mg/kg on rats with model of toxic impairment with aluminii chloride and found that rivastigmine dose-dependently increased locomotor activity, learning and memory.

The low dose of rivastigmine was beneficial to acquisition and also improved retention deficits of transgenetic male mice in water maze (Van Dam et al., 2005). The effects of rivastigmine on hippocampal-dependent memory in rodent models have not been enough studied. On the basis of the available findings, however, we may speculate that rivastigmine may be effective within a small range of doses (Yuede et al., 2007).

Our results allow us to conclude that Rivastigmine improves better learning than memory processes and this effect is also stronger in smaller dose applied. Both doses have similar stimulating effect on exploratory activity in rats.

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