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Exploratory activity in rats with scopolamine model of cognitive dysfunction treated with cholinesterase inhibitors

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Abstract

The aim was to study the effects of rivastigmine and metrifonate on the exploratory activity of rats with scopolamine model of cognitive dysfunction in "Activity cage" apparatus with photo-sensors (Ugo Basile, Italy). The scopolamine group with cognitive dysfunction decreased horizontal and vertical locomotor activity. Rivastigmine in a small dose can improve only the horizontal activity. Metrifonate, irrespective of the dose applied, improves the horizontal and vertical exploratory activity in rats in a significantly higher degree.

Keywords**:** cholinesterase inhibitors, scopolamine model, rats, exploratory activity, activity cage

Introduction

Dementia is a clinical diagnosis established on the basis of progressive cognitive decline. The most common types dementia are Alzheimer's disease (50-75%) followed by vascular dementia (20%), dementia with Lewy bodies (5%) and frontotemporal lobar dementia (5%) [1]. With the probable exception of vascular dementia, all involve a pathological accumulation of a native protein: in case of Alzheimer's disease (AD) it is the extracellular plaques of amyloid and intracellular tangels of hyperphosphorylated tau; in dementia with Lewy bodies (DLB) it is alpha-synuclein; in frontotemporal lobar dementia (FTLD) TDP-43 (transactive response DNA-binding protein 43 KDa) and holmark proteins for AD and DLB [2].

The pharmacological management of AD includes cholinesterase inhibitors (ChEIs) (tacrine, donepezile, galantamine, rivastigmine) and partial N-methyl-D-aspartate receptor antagonist memantine [3]. The main aim of ChEIs treatment is to inhibit enzymatic degradation of the neurotransmitter acethylcholine, resulting in an increased amount of acetylcholine in the synaptic terminals [4].

Rivastigmine belongs to second generation ChEIs, penetrate the central nervous system and increases the local concentration of Ach, more effective and less toxic in comparison to first generation ChEIs [5]. It is distinct from other available cholinesterase inhibitors (donepezil and galantamine) in that it is a pseudo-irreversible inhibitor of both acethylcholineaterase and butyrylcholineaterase [6]. The investigations of the effect of rivastigmine on neuropreservation, using a novel neurodegenerative primary rat cerebrovascular culture system, suggest that changes in metabolic activity that result from rivastigmine treatment are associated with increased neuronal survival [7]. Recent studies suppose that rivastigmine has also anti-inflammatory effects in mice with ulcerative colitis, rats with Crohn's disease [5] and can also ameliorate immunological and pathological parameters of experimental autoimmune encephalomyelitis in mice [8]. Inflammation plays also key role in pathogenesis of AD. It is the secondary event to the deposition of Aβ in the brain. Probably it is defense response to the damaging effects of Aβ connecting with neuronal degeneration, activation of microglia, release

Metrifonate is an organophosphorous compound used in clinical practice for treatment of schistosomiasis [10] and ectoparasite infestations on fish cultures [11]. Metrifonate has been used as an antihelminthic in tropical countries for more than 30 years, and even recently has been suggested as a treatment for dementias [12]. It is prodrug of the long-acting cholinesterase inhibitor dichlorfos. Metrifonat is converted by non-enzymatic process in long-acting cholinesterase inhibitor 2,2-dichlorovinyl dimethyl phosphate, its active metabolite [13]. Metrifonate well influence cognitive symptoms in AD with gradual onset of action and permanently maintained high levels of cholinesterase inhibition. Currently it is not used in clinical practice because data form 6-months duration clinical trials indicate two extremely rare but serious side effects such us respiratory paralysis and problems with neuromuscular transmission [14]. They probably are a result of prolonged inhibition of enzyme butyrylcholinesterase which is not well investigated in comparison of the enzyme acethylcholinesterase [15].

Scopolamine-treated rats have been widely used as a psychopharmacological model of chemically induced amnesia on which evaluated the efficacy of acetylcholinesterase inhibitors in the treatment of Alzheimer's disease. Scopolamine acts by blocking muscarinic cholinergic receptors and causes in young animal memory deficits similar to those seen in aging. More recent studies suggest that the mechanisms of this model are more complex and include in addition to cholinergic and gamma-aminobutyric and glutamatergic neurotransmitter systems [16].

The results of previous our study indicates that suppressive effect of scopolamine on learning and memory in rats is not dose dependent. The most pronounced suppressive effect on cognitive functions cause the dose of 1 mg/kg [17].

The aim was to study the effects of rivastigmine and metrifonate on the exploratory activity of rats with scopolamine model of cognitive dysfunction.

Materials and Methods

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. Scopolamine hydrobromide (Sigma). Metrifonate (Bayer) is 2,2,2-trichloro-l-hydroxy-ethyl)phosphonic acid dimethyl ester. Rivasrigmine (Novartis Pharma) is (S)-N-ethyl-3-[(1-dimethyl-amino) ethyl]-N-methyl-phenylcarbamate hydrogentartrate.

Animals

Male Wistar rats weighting 220-240 g were divided into 7 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 following experimental groups were used: Group 1: saline 0.1 ml/100g body weight (controls); Group 2: scopolamine hydrobromide 1 mg/kg (model group) + saline 0.1 ml/100g body weight; Group 3: scopolamine hydrobromide 1 mg/kg + rivastigmine 1 mg/kg; Group 4: scopolamine hydrobromide 1 mg/kg + rivastigmine 2 mg/kg; Group 5: scopolamine hydrobromide 1 mg/kg + metrifonate 30 mg/kg; Group 6: scopolamine hydrobromide 1 mg/kg + metrifonate 50 mg/kg and Group 7: scopolamine hydrobromide 1 mg/kg + metrifonate 80 mg/kg. Pre-treatment once daily was done with duration 11 days. On 12^{th} day the compounds were cholinesterase inhibitors were administered per orally 60 minutes before testing. The scopolamine was injected intraperitoneally 30 minutes before testing.

Behavioral test - Locomotor activity (Activity cage)

The horizontal and vertical activity in individual rats was registered by Ugo Basile Activity cage. The apparatus consist of an animal cage (with transparent cover) and an electronic unit. The activity detection relies on horizontal sensors, designed for the assessment of the ambulatory activity. The movements the animal makes counted and recorder by the electronic unit. Data related to horizontal and vertical activity are printed in digital form at pre-set intervals. The

activity recorded for 5 minutes, starting after placing the animal into the test cage. The locomotor measurements were performed between 08:00 and 12:00 in a quiet room under normal laboratory lighting.

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. $P<0.05$ was considered as significant.

Results and Discussion

In locomotor activity test control rats showed a high number of relative units compared to all studied groups on horizontal activity (Figure 1). The group treated with 1 mg/kg scopolamine and saline significantly decreased the number of relative units ($p<0.05$) on horizontal locomotor activity compared to the control group, treated with saline only (Figure 1). The rats with scopolamine and rivastigmine 1 mg/kg significantly increased the number of relative units ($p<0.05$) on horizontal activity compared to the group with scopolamine and saline (cognitive dysfunctions model group). The animals with scopolamine and rivastigmine 2 mg/kg did not change the number of relative units on horizontal movements in comparison with scopolamine model group only (Figure 1). The animals treated with scopolamine and saline decreased also vertical movement activity but without significance in comparison with control group. The rats with scopolamine and rivastigmine at dose of 1 mg/kg showed increased number of relative units

on vertical activity in comparison not only with scopolamine model group but also with control rats. The group with scopolamine and rivastigmine 2 mg/kg decreased the number of relative units on vertical activity compared with scopolamine group (Figure 1).

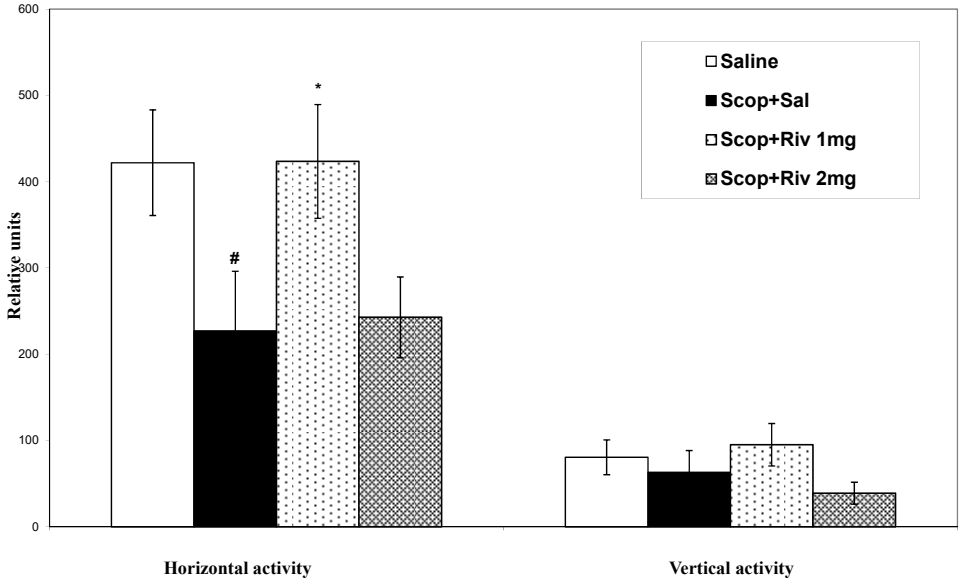

Figure 1. Effects of cholinesterase inhibitor *rivastigmine* on the exploratory activity in rats with scopolamine model of cognitive dysfunction.

$^{\#}p<0.05$ compared to the control group;

$^{*}p<0.05$ compared to the group with scopolamine model only.

The rats with scopolamine model of cognitive dysfunction significantly decreased the number of relative units ($p<0.05$) on horizontal activity compared to the saline group (Figure 2). The group treated with scopolamine and metrifonate at a dose of 30 mg/kg significantly increased the number of relative units on horizontal activity ($p<0.05$) compared to the saline group (control) and the scopolamine model group. The experimental group with scopolamine and metrifonate at a dose of 50 mg/kg increased the number of relative units ($p<0.05$) on horizontal moving activity in comparison with model of cognitive dysfunction group only. The animals with scopolamine and 80 mg/kg metrifonate showed the highest horizontal exploratory activity ($p<0.05$) in comparison with controls and ($p<0.01$) in comparison with scopolamine model group (Figure 2).

The three groups with scopolamine and metrifonate 30, 50 or 80 mg/kg increased the number of relative units on vertical activity compared to the control group but statistically significant differences were observed only at dose of 80 mg/kg ($p<0.05$). The same experimental groups of rats significantly increased the number of relative units ($p<0.05$) compared to the group treated with scopolamine and saline (Figure 2).

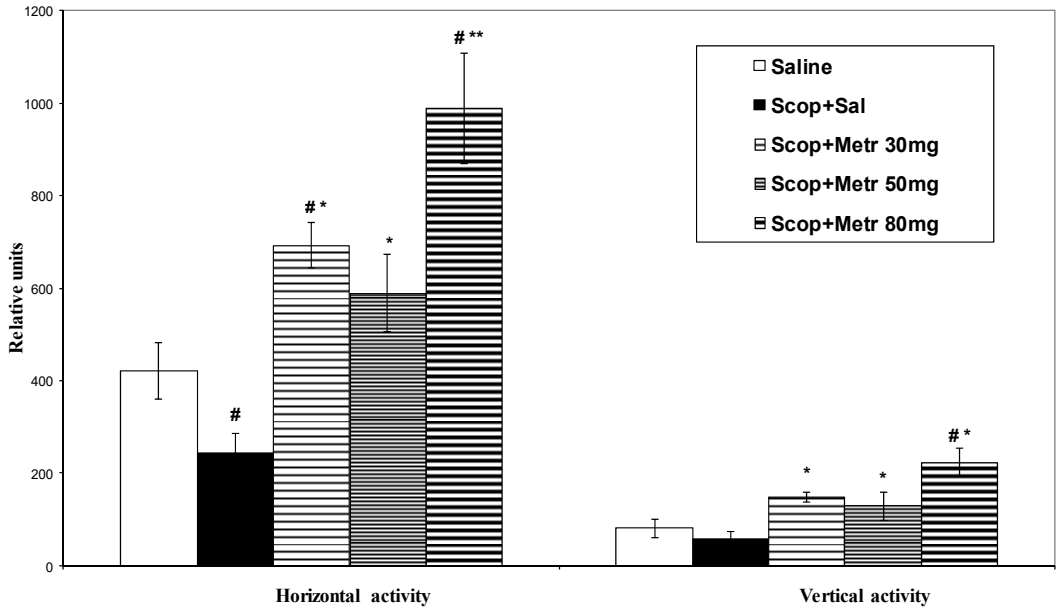

Figure 2. Effects of cholinesterase inhibitor *metrifonate* on the exploratory activity in rats with scopolamine model of cognitive dysfunction.

$^{\#}p<0.05$ compared to the control group;

$^{*}p<0.05$ and $^{**}p<0.01$ compared to the group with scopolamine model only.

Figure 3. Comparison the cholinesterase inhibitors *rivastigmine* and *metrifonate* on the exploratory activity in rats with scopolamine model of cognitive dysfunction.

$^{\#}p<0.05$ compared to the control group;

$^{*}p<0.05$ and $^{**}p<0.01$ compared to the group with scopolamine model only.

Figure 3 presents the summarized data from Figures 1 and 2 on horizontal and vertical locomotor activity in rats with scopolamine model of cognitive dysfunction in order to visually compare and discuss the effects of the studied cholinesterase inhibitors rivastigmine and metrifonate.

The cholinergic system plays a pivotal role in process of learning and memory therefore it underlies the pathogenesis of diseases connected with cognitive deficits. Blockade of muscarinic receptors in the target structures produces similar behavioral deficits [18]. Declarative memory in humans is the equivalent of episodic-like memory in rodents. Spatial memory formation represents a simple form of episodic-like memory in rodents that engages the basal forebrain cholinergic system and its target structures [19].

In connection with the suppressing effect of scopolamine on the central nervous system (CNS), it can be assumed that it not only suppresses cognitive functions, but also reduces the motor activity of the experimental animals. Our experiments confirmed this suggestion. We have found that this effect is more pronounced on the horizontal movements than on the vertical. Cholinesterase inhibitors have a stimulating effect on the CNS and can antagonize the depressant effects of scopolamine by increasing acetylcholine levels in the brain. Our results partially confirmed this assumption. Rivastigmine managed to antagonize the effects of scopolamine and to restore the horizontal and vertical motor activity of rats. This effect was not dose-dependent. It was markedly pronounced in the lower dose rivastigmine. Rivastigmine at a dose of 1 mg/kg completely restores the horizontal and vertical exploratory activity of the experimental animals. It is interesting, that a twice higher dose of rivastigmine does not remove the suppressive effect of scopolamine on the horizontal activity. Furthermore, rivastigmine at a dose of 2 mg/kg administered to the rats with scopolamine-induced cognitive dysfunction will additionally slow down the vertical movements.

Metrifonate in all studied doses (30, 50 and 80 mg/kg) antagonized the suppressing effect

of scopolamine on the horizontal and vertical locomotor activity of experimental animals. Although this effect is not dose-dependent, it is most pronounced at the highest dose. Moreover, metrifonate can stimulate exploratory activity of animals both horizontal and vertical. The rats with scopolamine model of cognitive dysfunction which received 80 mg/kg metrifonate showed an approximately twice higher locomotor activity compared to the controls.

Data reported by van der Staay and Booger [20] indicate that metrifonate at a dose of 60 mg/kg can antagonize the scopolamine-induced spatial memory deficits in cone field orientation task in rats. The cone field would be a useful component of a behavioral screening battery to test the effects of putative cognition enhancers. Experimental metrifonate administered chronically for 6 months at a dose of 100 mg/kg to 7-month-old doubly transgenic APP+PS1 mice reduced swimming speed and locomotor activity in both genotypes [21].

Data from our previous experiments (shuttle box test for active learning with negative reinforcement in naïve rats) showed improvement effect of rivastigmine on the process of learning and memory. In this test, we recorded a number of intertrial crossings, which is an indicator of horizontal locomotor activity. Both doses of rivastigmine (1 and 2 mg/kg) had a similar stimulating effect on the exploratory activity in naïve rats [22]. The study of the exploratoty activity of rats with a model of sodium nitrite induced hypoxia and metrifonate showed that only the 80 mg/kg metrifonate dose enhances the horizontal and vertical locomotor activity [23].

Conclusion

Comparing rivastigmine (second generation cholinesterase inhibitor for the treatment of mild to moderate Alzheimer's disease) with metrifonate (still under investigation) we found that metrifonate not only removes the suppressing effect of scopolamine but increases the horizontal and vertical activity of the animals to a degree that it is much higher than the controls.

It can be concluded that rivastigmine in a small dose can improve only the horizontal activity. Metrifonate, irrespective of the dose applied, improves the horizontal and vertical exploratory activity in rats in a significantly higher degree.

We expect the resulting experimental data can be useful in clinical practice by the fact that rivastigmine can be administered at lower doses to improve cognition without disturbing the physical activity of the patient. Moreover, if metrifonate be comes approved as a cholinesterase inhibitor for the treatment of Alzheimer's disease, it should be borne in mind that it will be particularly suitable for people with delayed motor function.

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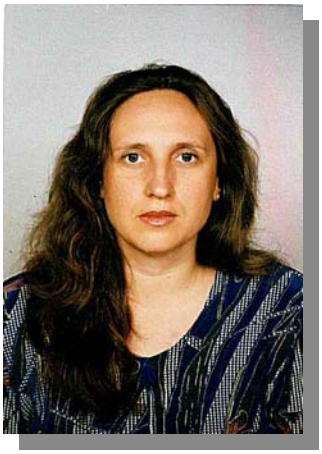

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