

EFFECTS OF CHOLINESTERASE INHIBITORS TACRINE AND METRIFONATE ON EXPLORATORY ACTIVITY IN RATS WITH INDUCED HYPOXIA

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ABSTRACT

Dementia is a common symptom of many degenerative brain diseases. The cholinesterase inhibitors are widely recommended for the treatment of Alzheimer's disease and different kind of dementia to improve memory functions. Tacrine is the first drug from this group. 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. Chronic application of sodium nitrite induced long-lasting effects such as hypoxia, neuron damage and impaired behavior.

The aim of our study was to study the effects of Tacrine and Metrifonate on exploratory activity in rats with induced hypoxia. The male Wistar rats (9 per group, with body weight 200-220 g) was treated with: 1st - Saline 0.1ml/100g body weight s.c. + Saline 0.1ml/100g body weight p.o. (controls); 2nd – Sodium nitrite 50 mg/kg s.c. + Saline 0.1ml/100g p.o., 3rd – Sodium nitrite 50 mg/kg s.c. + Tacrine 0.5 mg/kg p.o.; 4th – Sodium nitrite 50 mg/kg s.c. + Tacrine 1 mg/kg p.o., 5th - Sodium nitrite 50 mg/kg s.c. + Metrifonate 30 mg/kg p.o., 6th - Sodium nitrite 50 mg/kg s.c. + Metrifonate 50 mg/kg p.o., 7th - Sodium nitrite 50 mg/kg s.c. + Metrifonate 80 mg/kg p.o. The effect of tacrine and metrifonate on exploratory activity of rats was studied in automatic set-up for horizontal and vertical activity "Activity cage" with photo-sensors (Ugo Basile, Italy). Time spent on the apparatus was 5 minutes. The comparison between groups made by Instat computer program using analysis of variance (ANOVA for repeated measurements).

In locomotor activity test control rats showed the highest number of relative units compared to all studied groups on horizontal and vertical activity. The group treated with sodium nitrite significantly decreased the number of relative units compared to saline group on horizontal and vertical activity. The groups with sodium nitrite and Tacrine increased the number of relative units on horizontal and vertical activity compared to sodium nitrite group. The group with sodium nitrite and 30 mg/kg Metrifonate increased the number of relative units on horizontal activity only. The rats with sodium nitrite and 50 mg/kg Metrifonate slightly increased horizontal and vertical activity compared to hypoxia group. The animals with sodium nitrite and 80 mg/kg Metrifonate significantly increased the number of relative units on horizontal and vertical activity.

Our results allow us to conclude that sodium nitrite induced hypoxia impaired brain functions, horizontal and vertical locomotor activity in the animals. Tacrine and metrifonate can partly to antagonize inhibitory effects of sodium nitrite on exploratory activity in rats. The effect of Tacrine is better than Metrifonate on moving activity in rats with hypoxia.

Key words: Tacrine, Metrifonate, rats, exploratory activity

INTRODUCTION

Dementia is a common symptom of many degenerative brain diseases. The cholinesterase inhibitors are widely recommended for the treatment of Alzheimer's disease (AD) and different kind of dementia to improve memory functions. Tacrine is the first drug in this group approved for oral administration in 1993 in USA for treatment of memory problems of AD. It was found that tacrine leads to the rare but fatal hepatotoxicity (Alfirevic et al., 2007). It is the first drug of choice for patients with AD since introduction of second generation cholinesterase inhibitors – donepezile, galantamine and rivastigmine (Adrenwale et al., 2010). 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 (Cerf et al., 1962). Metrifonate well influence cognitive symptoms in AD with gradual onset of action and permanently maintained high levels of cholinesterase inhibition

(Lopez-Arrieta and Schneider, 2006). 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 (Jann, 1998). Currently it is not used in clinical practice because data from 6-months duration clinical trials indicate two extremely rare but serious side effects such as respiratory paralysis and problems with neuromuscular transmission (Lopez-Arrieta and Schneider, 2006). They probably are a result of prolonged inhibition of enzyme butyrylcholinesterase which is not well investigated in comparison of the enzyme acetylcholinesterase (Pohanka, 2011).

Chronic application of sodium nitrite (NaNO_2) induced long-lasting hypoxia, neuron damage and impaired behavior (Hlinak and Krejci, 1990).

The **AIM** of our study was to study the effects of Tacrine and Metrifonate on exploratory activity in rats with induced hypoxia (the model of aging brain).

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.

Compounds

Sodium nitrite (NaNO_2), Polfa.

Tacrine (Sigma) is (1,2,3,4-tetrahydro-5-aminoacridine).

Metrifonate (Bayer, Germany) is 2,2,2-trichloro-1-hydroxy-ethyl)phosphonic acid dimethyl ester.

Animals

Male Wistar rats weighting 200-220 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: 1st - Saline 0.1ml/100g body weight s.c. + Saline 0.1ml/100g body weight p.o. (controls); 2nd - Sodium nitrite 50 mg/kg s.c. + Saline 0.1ml/100g p.o. (hypoxia model group); 3rd - Sodium nitrite 50 mg/kg s.c. + Tacrine 0.5 mg/kg p.o.; 4th - Sodium nitrite 50 mg/kg s.c. + Tacrine 1 mg/kg p.o.; 5th - Sodium nitrite 50 mg/kg s.c. + Metrifonate 30 mg/kg p.o.; 6th - Sodium nitrite 50 mg/kg s.c. + Metrifonate 50 mg/kg p.o.; 7th - Sodium nitrite 50 mg/kg s.c. + Metrifonate 80 mg/kg p.o. Pre-treatment once daily was done with duration 11 days. On 12th day the compounds were administered 60 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

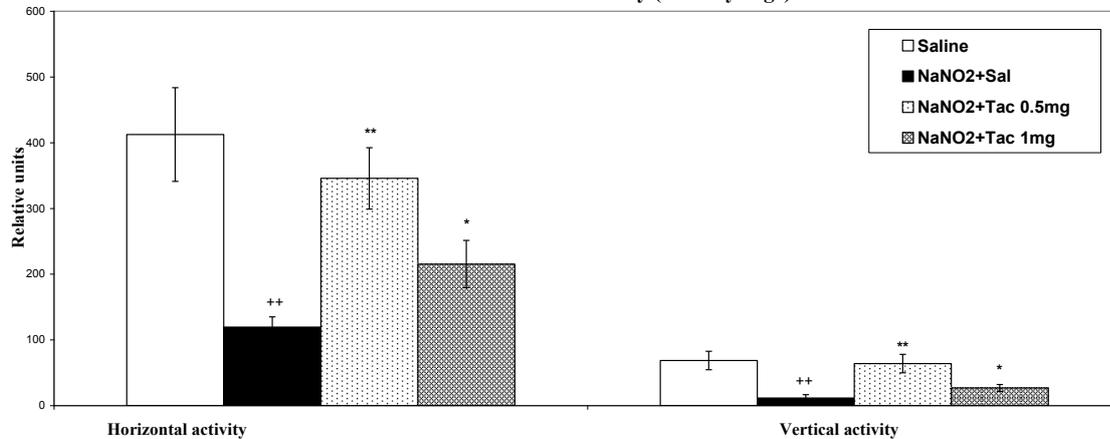
The means \pm SEM for each group of rats were calculated using Instat computer program. A two-way ANOVA for repeated measurements was used to compare different groups with the respective controls with the Turkey-Kramer multiple comparison test.

RESULTS

In locomotor activity test control rats showed the highest number of relative units compared to all studied groups on horizontal and vertical activity (Figure 1). The group treated subcutaneously with 50 mg/kg sodium nitrite and per os with saline significantly decreased the

number of relative units on horizontal ($P<0.01$) and vertical ($P<0.01$) locomotor activity compared to control group treated with saline only (Figure 1).

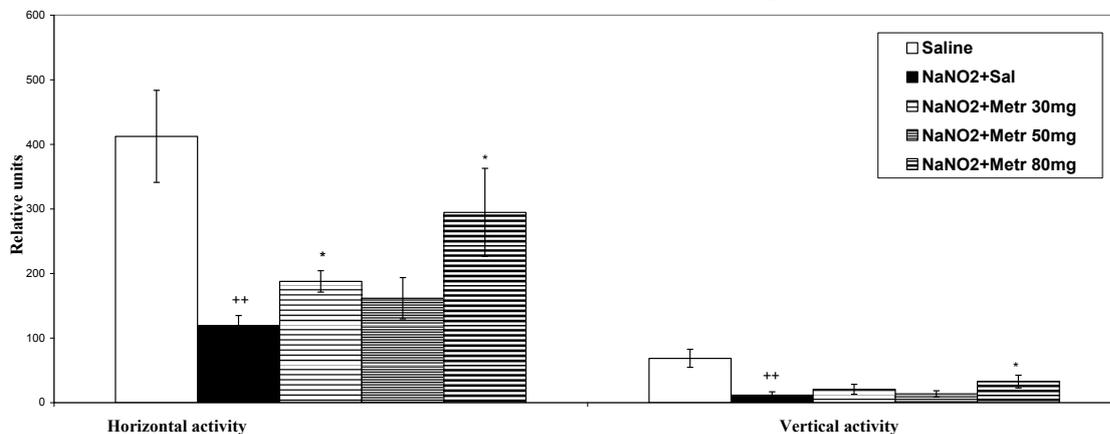
Figure 1. Effects of anticholinesterase drug Tacrine on hypoxia model of amnesia in rats
Locomotor activity (Activity cage)



⁺ $p<0,05$ and ⁺⁺ $p<0,01$ compared to the saline group (control);
^{*} $p<0,05$ and ^{**} $p<0,05$ compared to the group with sodium nitrite and saline (amnesia model).

The rats with sodium nitrite and Tacrine 0.5 mg/kg significantly increased the number of relative units on horizontal ($P<0.01$) and vertical ($P<0.01$) activity compared to the group with sodium nitrite and saline (hypoxia model group). The animals with sodium nitrite and Tacrine 1 mg/kg increased statistically significant ($P<0.05$) the number of relative units on horizontal and vertical movements in comparison with hypoxia model group (Figure 1).

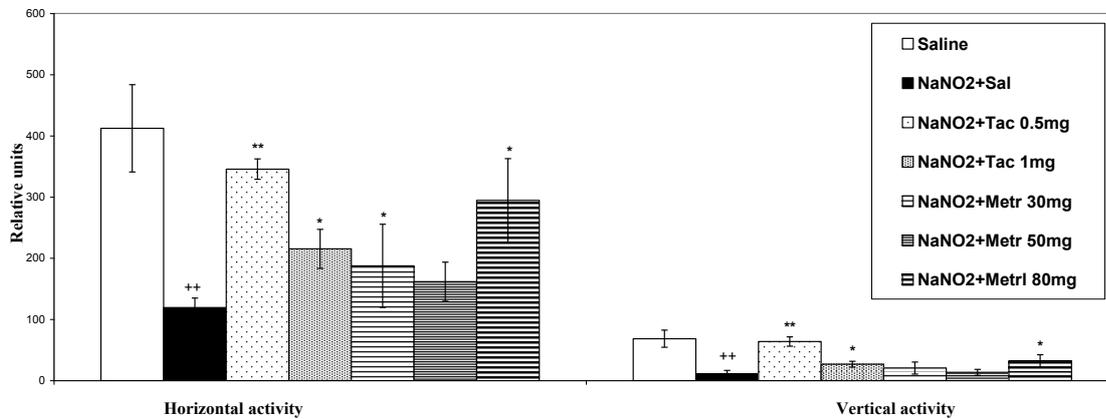
Figure 2. Effects of anticholinesterase drug Metrifonate on hypoxia model of amnesia in rats
Locomotor activity (Activity cage)



⁺ $p<0,05$ and ⁺⁺ $p<0,01$ compared to the saline group (control);
^{*} $p<0,05$ compared to the group with sodium nitrite and saline (amnesia model).

The rats with sodium nitrite and 30 mg/kg Metrifonate significantly increased the number of relative units ($P<0.05$) on horizontal activity only compared to the amnesia model group (Figure 2). The animals with sodium nitrite and 50 mg/kg Metrifonate slightly increased horizontal and vertical activity compared to group with sodium nitrite and saline. The animals with sodium nitrite and 80 mg/kg Metrifonate significantly increased the number of relative units on horizontal ($P<0.05$) and vertical ($P<0.05$) locomotor activity compared to hypoxia model group (Figure 2).

Figure 3. Comparison the effects of anticholinesterase drugs Tacrine and Metrifonate on hypoxia model of amnesia in rats - Locomotor activity (Activity cage)



⁺ $p < 0,05$ and ⁺⁺ $p < 0,01$ compared to the saline group (control);

^{*} $p < 0,05$ and ^{**} $p < 0,05$ compared to the group with sodium nitrite and saline (amnesia model).

In locomotor activity test Figure 3 shows the summarized data from Figures 1 and 2 on horizontal and vertical activity in rats with hypoxia model of amnesia in order to visually compare and discuss the effects of studied cholinesterase inhibitors Tacrine and Metrifonate.

DISCUSSION

There is data that chemical hypoxia by sodium nitrite induced changes in spontaneous behavior in rats and structure changes in CNS after a few days application (Hlinak et al., 1990). Our results confirmed this suggestion. The chronic cerebral hypoxia leads to severely reduced exploratory activity of both the horizontal and vertical movements in rats. The anticholinesterase drug Tacrine may antagonize the damaging effect of the sodium nitrite and enhance locomotor activity in animals. This effect was dose-independent. In our experiments the smaller dose showed much better results. We found that Metrifonate at all tested of us doses has a more pronounced effect on horizontal locomotor activity of the rats. The highest dose Metrifonate showed the best results on horizontal and vertical exploratory activity in experimental animals. The effect of Metrifonate was also dose-independent. The middle dose slightly affected locomotor activity in rats. Interesting results were obtained when compare the effects of the two cholinesterase inhibitors is presented in Figure 3. Tacrine has the better improving effect on horizontal and vertical locomotor activity in rats with sodium nitrite induced hypoxia in comparison with metrifonate. The best improving effect on exploratory activity in rats has Tacrine in dose 0.5 mg/kg. The dose of 80mg/kg Metrifonate has similar but less pronounced effect in this model of amnesia.

The sodium nitrite is considered inhibitor of protein synthesis that is known to cause amnesia (Vohra and Hui, 2000). It is used as a pharmacological model of anterograde and retrograde amnesia to investigate the effects of different compounds on CNS (Kishore and Singh, 2005; Ojha et al., 2010). Cholinesterase inhibitors Tacrine and Metrifonate increase the levels of acetylcholine in the brain areas associated with learning and memory such as the hippocampus and the brain cortex. They have improving effects of cognitive functions and are used for the treatment of Alzheimer's dementia and age-related damages in brain functions (Quian et al., 2012). Recent studies of Metrifonate in doses of 60 mg to 120 mg in the cortex and liver of rats showed that it not only inhibits acetylcholinesterase, but oxidative stress influences and regulates the process of apoptosis (Pohanka et al., 2011). Prolonged cholinesterase inhibition and the related improving effect of Metrifonate on cognitive brain functions in support of proposal for its use as a treatment of AD. The creation of new less toxic analogs of Tacrine is evidence of continued interest to this

medicine. It is expected that in the future they could replace it in clinical practice (Tumiatti et al., 2010).

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