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Rivastigmine affects Memory Disturbances in Rats with Abdominal Hypertension

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Abstract

The aim of our study was to investigate whether artificially increased intra-abdominal pressure (IAP) in rats lowers their cognitive potential and Rivastigmine is able to diminish this disturbance. Three groups of male Wistar rats were used: 1st saline; 2nd IIAP to 25mmHg and 3rd IAP + Rivastigmine. In shuttle-box test the following parameters was observed: number of avoidances, escapes and intertrial crossings. Saline group increased the number of avoidances on learning session and kept it in memory retention. The group with IAP decreased the avoidances and intertrial crossings during learning and memory tests. The group with model of IAP and rivastigmine increased avoidances and intertrial crossings on

learning and memory tests compared to group with model. Our results allow us to conclude that high intra-abdominal pressure impairs learning and memory processes in rats. Rivastigmine improves cognitive functions in rats with intra-abdominal and subsequent cerebral hypoxia.

Keywords : Rivastigmine, memory disturbances, rats, abdominal hypertension, shuttle-box

Introduction

Increased intra-abdominal pressure (IAP) affects organ function in critically ill patients and may lead to abdominal compartment syndrome (ACS). Even slight increase in intra-abdominal pressure has negative influence on the respiratory, cardiovascular, cerebral, gastrointestinal, hepatic, and renal functions. Intra-abdominal hypertension causes visceral organ hypoperfusion, intestinal ischemia and may also lead to bacterial translocation, release of cytokines and production of free oxygen radicals. All these factors may contribute to the development of multiple organ failure.

Current studies shown that increased intra-abdominal pressure modulate brain perfusion induced hypoxic changes [1,2] and impair memory and other cognitive functions. Acetylcholinesterase inhibitors restore the central cholinergic deficit and have modest but significant improving action on cognitive impairments. They improve the learning and memory processes in Alzheimer's disease [3]. The second generation cholinesterase inhibitors (donepezil, rivastigmine, galantamine) and N-methyl D-aspartate receptor antagonist memantine have been widely prescribed and studied [4]. The rationale for the use of cholinesterase inhibitors in the treatment of dementia 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 [5].

Rivastigmine is novel carbamate-based, reversible, noncompetitive inhibitor of acethylcholinesterase (AChE) and butyrylcholinesterase (BChE) [6, 7]. Although rivastigmine inhibits both AChE and BChE, it is the only available cholinesterase inhibitor that appears to be further relatively selective for the postsynaptic G1 monomer form of AChE

in the central nervous system to areas of the cortex and hippocampus. Moreover, rivastigmine not metabolize by the hepatic microsome system. Thus, it is unlikely to have significant pharmacokinetic interactions with other medications [8]. Comparative study on the effects of 8 holinesterase inhibitors (tacrin, bis-tacrin, donepezil, rivastigmine, galantamine, heptil-phisostigmin, TAK-147 and metrifonate) on the activity of AChE and BChE in human brain cortex found that only rivastigmine is high specific for BChE [9]. There is scientific evidence that observed cognitive improvement depends of the level of AChE inhibition. It is not quite studied the role of BChE on the cognitive brain functions [10]. Current studies on neurodegeneration model of fetal rat primary cortical cultures present neuroprotective and neurorestoring effect of rivastigmine and its ability to improve neuronal morphology and to restore synaptic function. The researchers assume that BChE-inhibition is a new therapeutic strategy for treatment of impaired learning and memory functions [11].

The aim of our study was to investigate whether artificially increased intra-abdominal pressure (IAP) in rats lowers their cognitive potential and Rivastigmine is able to diminish this disturbance.

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 and Ethics Committee of the Medical University Plovdiv.

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 8. 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) (control group); B: increased intraabdominal pressure (IAP) (model group) and C: increased intraabdominal pressure (IAP) + Rivastigmine 1.0 mg/kg.

Rat model of increased intraabdominal pressure (IAP)

The preliminary preparation of the rats from the model group was performed as follows:

- Anaesthetizing (Xylazine 2% - 10 mg/kg + Ketamine (Calipsol) 5% - 100 mg/kg).
- Fixing the animal on a polystyrol table;
- Creating a pneumoperitoneum using a cannula and fixing the latter onto the skin.

The elevation of IAP of the experimental group of rats was achieved by means of a pump and included the following stages:

- Coupling an elevated pressure system to the cannula and gradually (10 min) elevating IAP up to 25/30 mm/Hg.
- Maintaining the elevated IAP for more than 120 min for the purposes of simulating an acute ACS.

The individual procedures were performed under conditions of continuous monitoring of anaesthesia depth, temperature, pulse and respiration.

Behavioral test

The active avoidance test with negative reinforcement was performed on a next day 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 performed using the same parameters without foot stimulation. The following behavioral sings were observed: number of correct responses on conditioned stimuli, i.e. avoidances; number of escapes from foot stimulation (unconditioned stimuli responses); 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. P<0.05 was considered as significant.

Results and discussion

In active avoidance test saline group increased the number of conditioned stimuli responses (avoidances) on 2nd, 3rd, 4th (p<0.05) and 5th (p<0.01) days of learning session and kept it in memory retention on 12th day (p<0.05) compared with 1st day (Figure 1). The rats with IAP significantly decreased the number of avoidances during learning session 2nd (p<0.05), 3rd (p<0.01), 4th and 5th (p<0.05) days compared to the same days saline group. On memory retention test the IAP-model group significantly decreased (p<0.05) the number of avoidances compared to the 12th day saline group. The rats with IAP and rivastigmine increased avoidances in 2nd (p<0.05), 3rd (p<0.01), 4th (p<0.05), 5th (p<0.01) days learning and on memory test (p<0.01) compared to rats with IAP-model only (Figure 1).

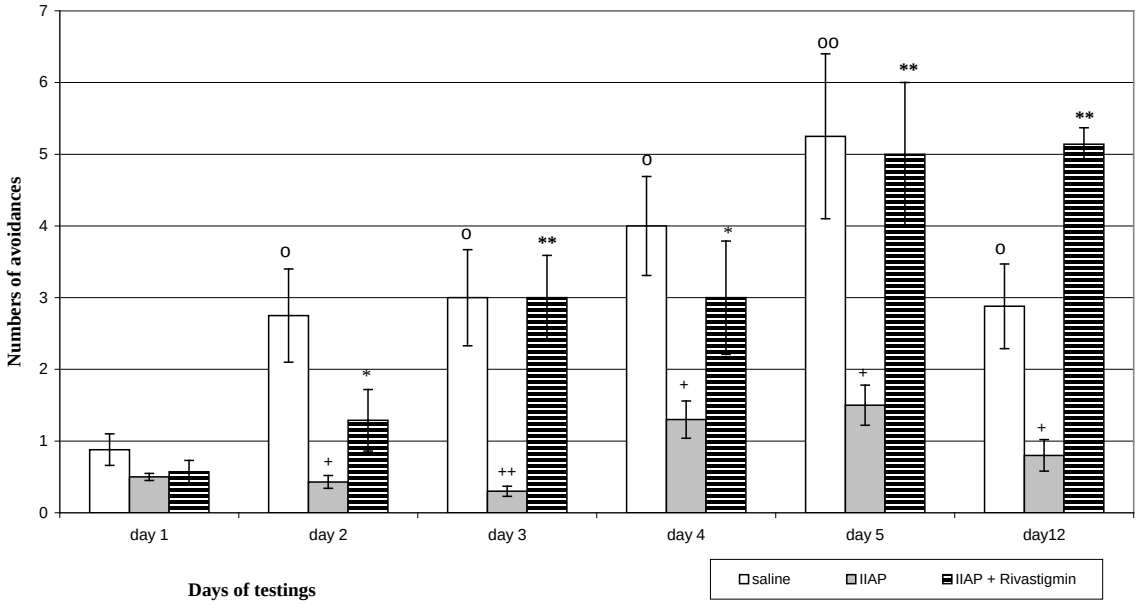

Figure 1. Effects of IAP and Rivastigmine on learning and memory processes in rats. Shuttle-box active avoidance test. Number of conditioned stimuli responses (avoidances). ^{o}p<0.05 and ^{oo}p<0.01 compared to the 1st day control group;$^{+}$p<0.05 and $^{++}$p<0.01 compared to the same day control group; *p<0.05 and **p<0.01 compared to the same day group with IAP.

In the shuttle-box test control group had no significantly change on the number of unconditioned stimuli responses (escapes) in the learning session and memory test (Figure 2). The IAP-model group showed the tendency to decrease the number of escapes on all learning days and memory retention, compared to the same day saline group, but it was significant only on 1st day learning ($p<0.05$). The rats with IAP treated with 1 mg/kg rivastigmine showed the tendency to increase the number of escapes on 5 days learning and memory retention, but it was significant on 3rd and 4th day learning ($p<0.05$), compared to the same days group with IAP-model only (Figure 2).

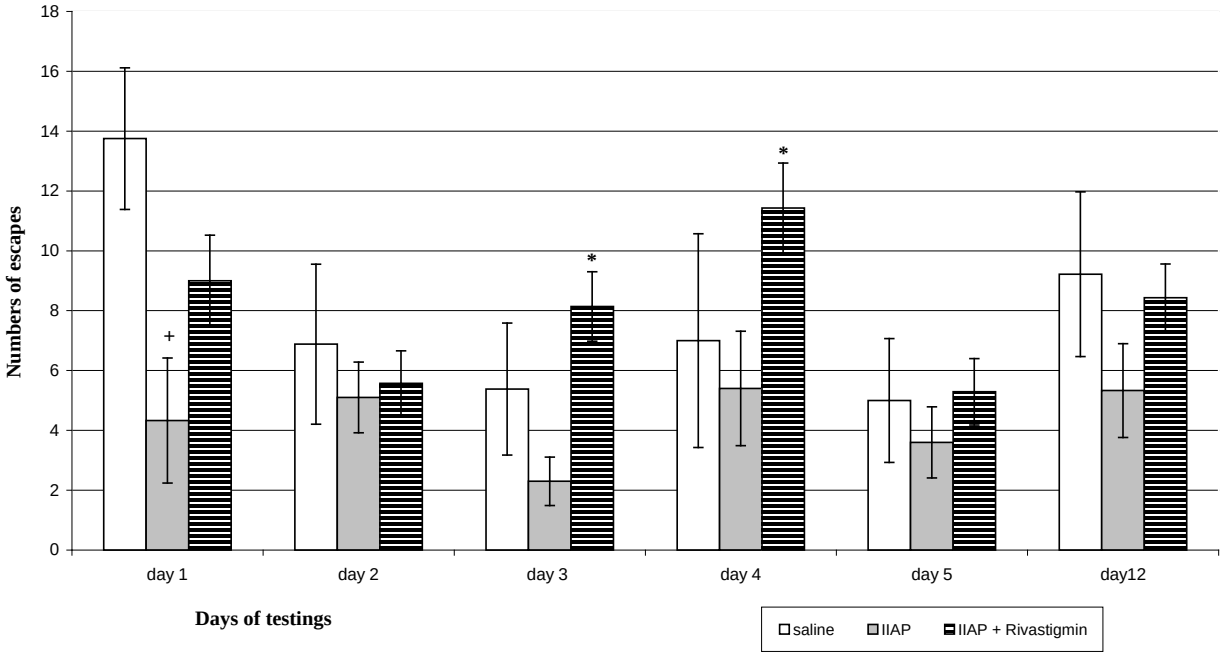

Figure 2. Effects of IAP and Rivastigmine on learning and memory processes in rats. Shuttle-box active avoidance test. Number of unconditioned stimuli responses (escapes). $^{+}p<0.05$ compared to the same day control group; $^{*}p<0.05$ compared to the same day group with IAP.

In active avoidance test with negative reinforcement control rats with saline did not changed significantly the number of intertrial crossings on learning session and memory test compared to the 1st day learning (Figure 3). The group with model of IAP showed the tendency to increase the intertrial crossings on learning session, but do not kept it on memory test compared to the saline group. The rats with IAP and 1 mg/kg rivastigmine showed the

tendency to increase the number of intertrial crossings on all days learning, but it was significantly increased on memory test ($p<0.01$), compared with same day the model group (Figure 3).

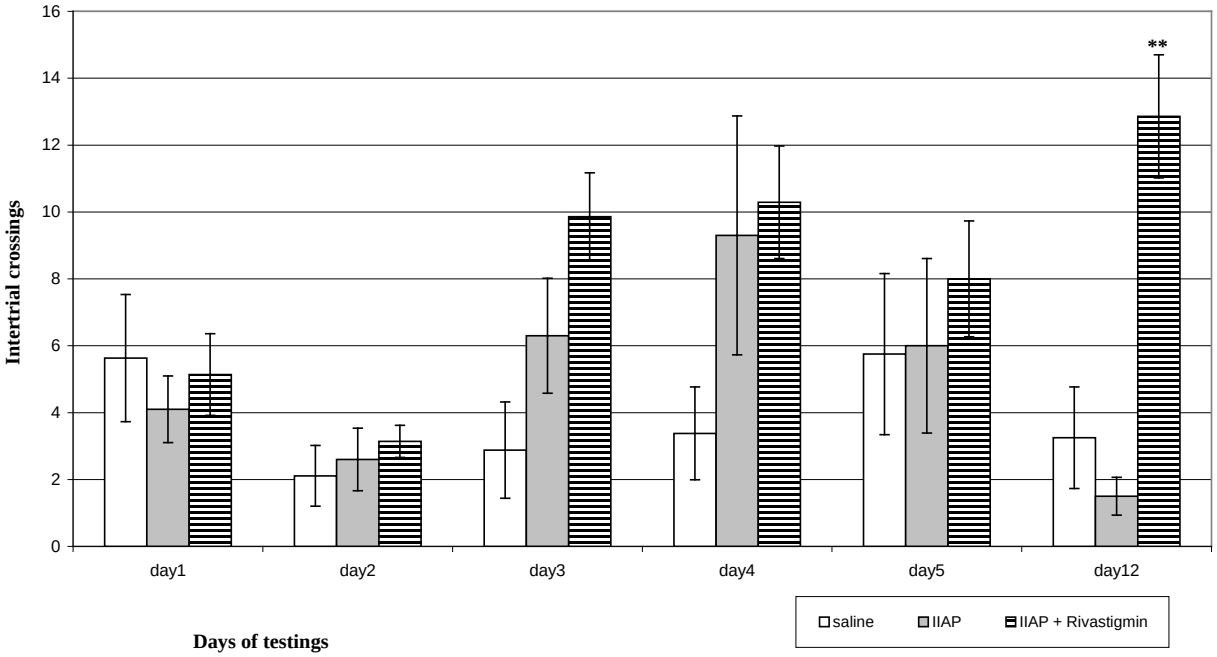

Figure 3. Effects of IAP and Rivastigmine on learning and memory processes in rats. Shuttle-box active avoidance test. Number of intertrial crossings. ,*p<0.05 compared to the same day group with IAP.

The animals with increased intra-abdominal pressure showed poor learning and impaired memory paradigm, compared to controls. Evidently this effect is due to the hemodynamic effects of increased abdominal pressure affecting the brain functions [2].

Rivastigmine at dose of 1 mg/kg antagonized the effect of IAP on the cognitive functions improving learning and memory. 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 a dose used by us increased locomotor activity (number of intertrial crossings) in experimental animals. Executive increasing locomotor activity have been observed in memory retention test only.

The disturbances in cognitive functions in IAP-experimental model are probably results from

brain hypoperfusion, cerebral and intestinal ischemia [2]. Activation of the efferent *n.vagus* by pro-inflammatory cytokines like IL-1β and TNF-α released from macrophages has been shown to attenuate inflammation in a mouse model of acute colitis [12] and in other cytokine mediated inflammatory conditions [13]. Pro-inflammatory cytokines can also reach the brain via the *area postrema* which lies in close proximity to the nucleus of the solitary tract and the *dorsomotor nucleus* of the *n.vagus* in the brainstem [14]. The cholinergic inflammatory pathway first described by Tracey and his colleagues [15]. Acetylcholine released from vagal nerve terminal may interact with α7-nAChR on macrophages or dendritic cells and the activity of this pathway may be deficient in intestinal diseases including chronic inflammation such as ulcerative colitis [15]. In support of this hypothesis are the data of Shifrin and colleagues [16]. Their study revealed that the cholinesterase inhibitor rivastigmine is able to reduce gastro-intestinal inflammation by action at various sites at which it preserves acethylcholine. They provided experimental evidence that rivastigmine (1-2 mg/kg) can significantly reduce overt signs and the accompanying structural and inflammatory changes of acute colitis induced in two experimental models in mice and rats which represent the pathophysiological features of ulcerative colitis and Crohn's disease. Acetylcholinesterase inhibitors can augment levels of acetylcholine in the brain to compensate for loss of cholinergic function [17]. Scali and colleagues [18] found 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.

The ischemic injury and inflammatory vascular peripheral disorders may induce brain disturbances with long-term consequences. The vascular damage patterns typically precede neuronal injury, an initial vascular insult to the brain elicited by hypoxia, hypoperfusion or a disrupted blood-brain barrier precedes observed amyloid pathology and cognitive decline in Alzheimer's disease [19].

Conclusion

The results of previous our experiments on naïve rats showed that rivastigmine

improves better learning than memory processes and this effect is also stronger in a dose of 1 mg/kg in comparison to 2 mg/kg. Both doses have similar stimulating effect on exploratory activity in rats [20].

The data of this study allow us to conclude that high intra-abdominal pressure impairs learning and memory processes in rats. The low levels of oxygen and glucose in the brain results in a significant decrease of ATP and increasing lactate production [21]. Rivastigmine improves cognitive functions in rats with intra-abdominal and subsequent cerebral hypoxia. It's probably the result of increased level of acetylcholine in synaptic terminals [22].

In clinical practice it is important to prevent patients from ischemic episodes and to treat them as soon as possible. Rivastigmine, the second generation cholinesterase drug, is not only for treatment of Alzheimer's disease. Experimental date present rivastigmine as a new therapeutic approach for patients with hypoxic changes in CNS and others organs result of increased intra-abdominal pressure and for treatment of disorders with immune-type inflammation such as ulcerative colitis and Crohn's disease.

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Author Contributions

The model of increased intra-abdominal pressure was performed in the Department of Medical Physics and Biophysics by Valentin Turiiski and Rayna Ardasheva. The behavioral test was conducted in the Department of Pharmacology and Clinical Pharmacology by Darinka Dimitrova and Damianka Getova.

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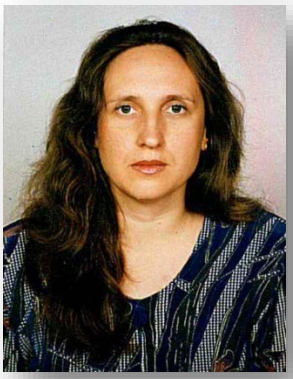

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