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Research Article

Effects of Rivastigmine on Behavioral Tests for Passive Training in Rats with Abdominal Hypertension

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Abstract: Intra-abdominal hypertension may increase brain venous pressure, which may lead to brain injury. Elevated intracranial pressure causes encephalopathy and the risk of neuronal damage due to the sharp decrease in cerebral perfusion pressure. Increased intra-abdominal pressure (IIAP) modulates brain perfusion, induces hypoxic changes and impairs memory and other cognitive functions. Rivastigmine, last introduced in clinical practice cholinesterase inhibitor, is a second generation carbamate-based, reversible, noncompetitive inhibitor of acethylcholinesterase and buthyrilcholinesterase, used for treatment of mild to moderate Alzheimer's disease. The aim was to investigate whether artificially IIAP in rats lowers their cognitive potential and Rivastigmine is able to diminish this disturbance using passive avoidance tests. Three groups of male Wistar rats were used: 1st saline 0.1ml/100g body weight; 2nd IIAP to 25mmHg; 3rd IIAP + Rivastigmine 1 mg/kg. Step-through and step-down passive avoidance tests were performed in originally made apparatus by Ugo Basile, Italy. In both tests was observed the latency of reactions – for the step-through time spend in the light chamber (178 seconds) and for the step-down time spend on the resting platform (60 seconds) of the apparatus. The rats with IIAP showed the latency of reaction similar than control group in both tests. In step-through test the group with model of IIAP and rivastigmine increased the latency of reaction on learning and long memory retention. In step-down tests the same group increased the latency only in long memory retention. It can be concluded that rivastigmine improves long term memory in rats with intra-abdominal and subsequent cerebral hypoxia.
Keywords: Rivastigmine, rats, abdominal hypertension, step-through, step-down.

INTRODUCTION

Elevation of intra-abdominal pressure (IAP) affects hemodynamics and can be crucial factor triggering organ injury due to tissue ischemia and oxidative stress [1].

Increased IAP may lead to abdominal compartment syndrome (ACS). Abdominal compartmant syndrome is defined by values of the IAP higher than 20 mm/Hg with evidence of multiple organ failure. Heavy pathological changes have been observed in lung, small intestine, large intestine, stomach, liver, kidney, spleen, and anterior abdominal wall in an experimental model of the ACS in rats [2]. 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.

Maintaining the IAP over 12 mmHg for 8 weeks caused increased oxidative damage to both lipids and proteins with an increased pro-oxidant-antioxidant balance. In attempt to compensate for this damage the muscle fibers increase their glutathione reductase and glutathione peroxidase activity [3]. Persistent intra-abdominal pressure significantly decreased portal vein flow in male Sprague-Dawley rats (two folds in IAP 6 mmHg for two hours). Histological examination made after 24 hours showed signs of liver damage – enhanced hepatocyte proliferation and apoptosis in 25% of animal [4]. Intra-abdominal bleeding plus intra-abdominal hypertension cause damage to the cardiovascular and respiratory functions and lead to ACS in rabbits [5]. Although the effects of increased IAP on the abdominal organs are well known, there is a limited data regarding its effects on other organs and systems in the body, for example the thyroid hormones. Secretion of vasopressin, adrenocorticotropic hormone and cortisol increases with increasing IAP [6].

Intra-abdominal hypertension decreases perfusion pressure [7] and has valuable effect at body

compartments with limited compliance such as cranial cavity. Several studies have reported increased intracranial pressure (ICP) as immediate sequence of IAP elevation [8]. According Monroe-Kelly doctrine [9] intracranial pressure rises rapidly due to inelasticity of the compartment causing lowered blood flow. Oxygen supply insufficiency produces encephalopathy and neuronal damage including memory processes disorder and cognitive malfunctions [10, 11]. Intra-abdominal hypertension increases brain venous pressure, which may lead to brain injury. Experiments with IAH in rats (25 mmHg for 90 minutes) indicate increased jugular venous pressure, decreased levels of total and ionized magnesium, increased calcium and zinc content in the brain. The histological data showed ischemic neuronal cell stress [12].

The AChE is the major cholinesterase within the human brain [13]. A part of pathogenesis of Alzheimer disease (AD), the most common type of dementia, represent degeneration of cholinergic projection from the nucleus basalis Meynerti to the forebrain neocortex and associated limbic structures [14]. During progression of the AD, BChE activity increased thus compensating the loss of AChE activity. BChE can substitute for AChE constitutively [13, 15]. Current drugs for the treatment of AD and other type of dementias for example vascular dementia and dementia associated with Parkinson's disease are non-selective (rivastigmine) and AChE-selective (galantamine and donepezil) inhibitors. Rivastigmine, last introduced in clinical practice cholinesterase inhibitor, is among the most important drugs investigated in 3-months and 6-monts clinical trials [16] with superior properties in term of specificity of action and low risk of adverse effects, which is approved for use in 60 countries including all member states of the European Union and the USA [17]. Rivastigmine is a second generation carbamate-based, reversible, noncompetitive inhibitor of AChE and BChE known for its ability to improve cognitive functions in Alzheimer's disease [18, 19]. Moreover, the role of BChE in the cognitive brain functions is still not quite studied. The researchers assume that BChE-inhibition is a new therapeutic strategy for treatment of impaired learning and memory functions [20, 21]. Currently, rivastigmine is the object of research and development of new experimental animal model of depression and cognitive impairments under chronic mild stress. It is found that cholinesterase inhibitors rivastigmine and donepezil have antidepressant-like effects and reduce the risk of cognitive decline [22].

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

MATERIAL AND METHODS

Ethical statement

All procedures used in this study were conducted in compliance with the rules and principles of the European 86/609/EEC Directive, and were approved by the Bulgarian Food Safety Agency and Ethics Committee of the Medical University Plovdiv.

Chemical compounds

Rats from IAH groups were anaesthetized by combination of xylazine (2% solution) -10mg/kg + ketamine (5% solution) -100mg/kg, injected intraperitoneally.

Rivasrigmine (S)-N-ethyl-3-[(1-dimethyl-amino) ethyl] -N-methyl- phenylcarbamate hydrogentartrate is a Novartis Pharma product.

Animals

The 24 mature male Wistar rats (age of 3 months, weight of 220–240 g) were divided into 3 groups of 8 animals. Rats were kept under standard laboratory conditions in a 12/12 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: control group A (n=8) – animals injected with saline (1 ml/kg body weight); model group B (n=8) – undergone artificial intra-abdominal hypertension (IAH) of 25 mmHg for 3 hours and group C (n=8) - undergone artificial IAH with identical parameters and consequently treated with rivastigmine 1.0 mg/kg. Pre-treatment once daily was done with duration 7 days. The compounds were were administered per orally 60 minutes before testing.

Rat model of increased intra-abdominal pressure (IIAP)

Artificially induced IAH was performed in following steps:

- The animals were anaesthetized according above mentioned procedure.
- The animals were fixed on electrical thermophore maintaining the temperature constant (+37°C) during the experiment.
- Modified percutaneous fixing of venflon was performed in order to induce pneumoperitoneum [23].
- Intra-abdominal pressure of animals was elevated by manual insufflation and performed at two stages: coupling of a high-pressure system with the venflon and gradually (for 10 minutes) increase the intra-abdominal pressure up to 25mmHg. The process was controlled by sphygmomanometer.

The increased intra-abdominal pressure had been maintained for time period of 180 minutes.

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

Behavioral tests

Step-through passive avoidance test

An automatic set-up for a passive avoidance "step-through" test (Ugo Basile, Italy) was used in a wire-floor cage with separate light and dark compartments. The test parameters were as follows: door delay for 6s, door opened to allow entry into darkened chamber for 12s, 0.4mA foot shock 9 sec later. Learning sessions were performed over 2 consecutive days, a short memory retention session was performed 24 hours later (3rd day), and a long memory retention session was performed on the 10th day. Memory retention tests were performed using the same parameters, except for the absence of a foot shock. Sessions consisted of 3 trials separated by 30 minute intervals. The learning criterion used was a latency of reaction of 180 ± 2 seconds in the light chamber.

Step-down passive avoidance test

An automatic set-up for a passive avoidance "step-down" test (Ugo Basile, Italy) was used in a wire-floor cage with round central plastic platform. Learning sessions consisted of 2 trials separated by a 60 minute interval. During each trial, electronic stimulation (0.4mA) was delivered to the wire floor for duration of 10s. Learning sessions were performed over 2 consecutive days, a short memory retention session was performed 24 hours later (3^{rd} day), and a memory retention session was performed on the 7^{th} day. Memory retention tests were performed using the same parameters, but with the absence of a foot shock. A latency of reaction of 60 s (the rat was required to remain on the platform for more than 60 s) was used as a criterion for learning and retention.

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

Effects of IIAP and rivastigmine on step-through passive avoidance test

The control group of rats showed a prolonged latency of reaction ($P<0.05$) during learning session and both short-term (3^{rd} day) and long-term (10^{th} day) memory tests when compared to Day 1 learning session performance (Figure 1). Animals with the IIAP showed a latency of reaction similar than the respective day the control rats on the 1^{st} and 2^{nd} learning sessions and memory tests. The group with IIAP and rivastigmine significantly increased ($P<0.05$) the time spend in the light chamber of the apparatus on the 1^{st} day learning and on the long memory performance ($P<0.05$) compared to the rats with increased intra-abdominal pressure only (Figure 1).

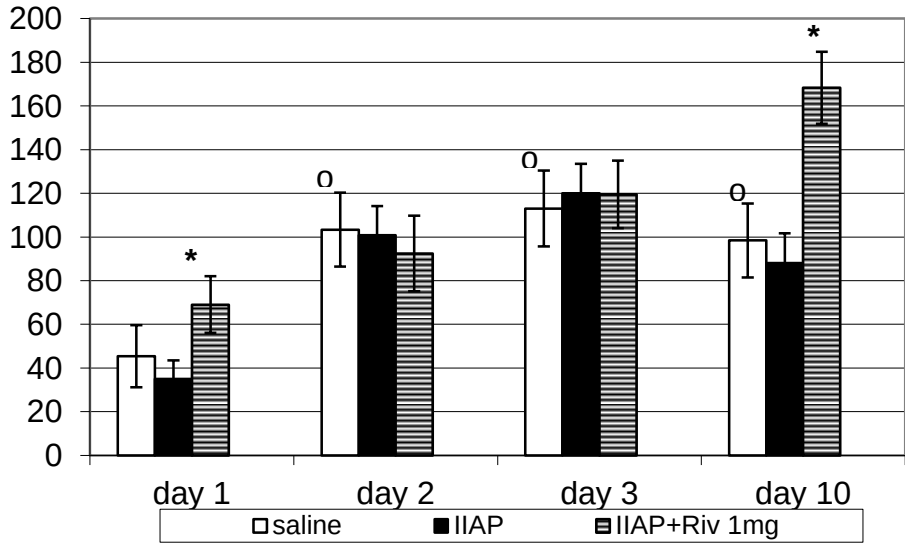

Fig-1: Effects of IIAP and rivastigmine on learning in memory in rats (Step-through passive avoidance test). X-axis – days of testing; Y-axis – latency of reaction in seconds;
$^{o}P<0.05$ compared to the first day control group;
***P<0.05 compared to the respective day IIAP model group.**

Effects of IIAP and rivastigmine on step-down passive avoidance test

The control group of rats demonstrated a prolonged latency of reaction ($P<0.05$) on the 2^{nd} day of learning and on both short-term (3^{rd} day) and long-term (7^{th} day) memory retention tests when compared to Day

1 performance (Figure 2). In the step-down passive avoidance test the rats with model of IIAP showed also the latency of reactions very close to the saline group. The rats with IIAP treated with rivastigmine showed a latency of reaction similar to control group and group with IIAP on both days of learning and short memory tests, but significantly prolonged the latency during long-term memory retention testing (P<0.05) when compared to the same day group with model of IIAP only (Figure 2).

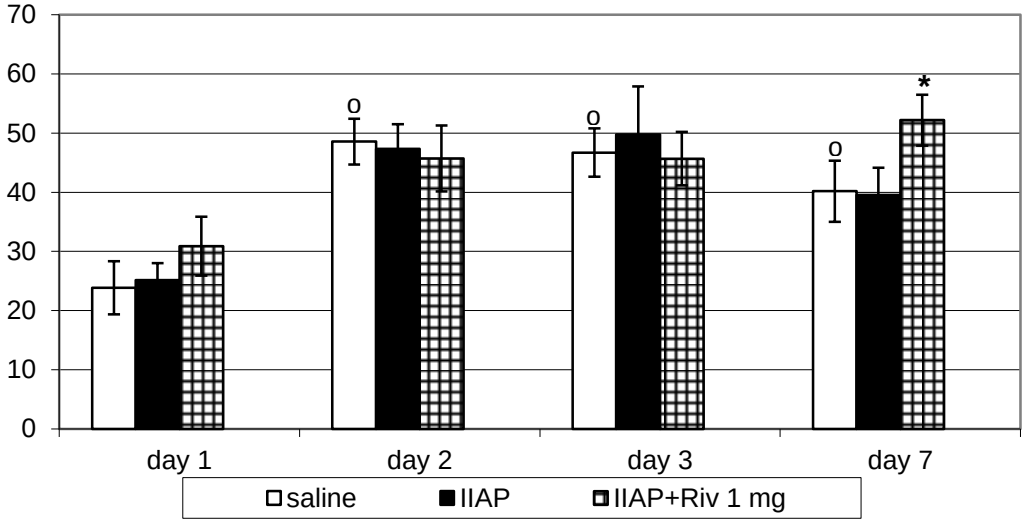

Fig-2: Effects of IIAP and rivastigmine on learning in memory in rats (Step-down passive avoidance test). X-axis – days of testing; Y-axis – latency of reaction in seconds;
^{o}P<0.05 compared to the first day control group;
***P<0.05 compared to the respective day IIAP model group.**

DISCUSSION

The control group improved training and formed long-term and short-term memory traces in both the test for passive training – step-through and step-down.

The results obtained in two tests for passive training allowed conclusion that IIAP have small impairing effect on cognitive functions in rats. The reason is probably in the design of the experiment because IIAP was performed once, seven days later was performed step-through test and 14 days after IIAP step-down test. Our previous data from the shuttle-box test showed that high IAP significantly affected cognitive functions. Deteriorating cognitive functions were observed for a week [24]. Hemodynamic changes induced by IIAP probably lead to cerebral hypoxia and impaired learning and memory. Acutely IIAP can lead to multisystem organ dysfunction. Increased intra-cranial pressures may cause brain dysfunction or aggravate head injury edema, venous thrombosis and thromboembolism [25]. Global cerebral ischemia/reperfusion injury encompasses complex pathophysiological sequelae, including loss of hippocampal neurons and behavioral deficits. Progressive neuronal death and memory dysfunctions culminate from several different mechanisms like oxidative stress, excitotoxicity, neuroinflammation and cholinergic hypofunction [26, 27].

The lack of significant difference between the control rats and rats with IIAP on learning and memory in the two tests of passive training, probably due to the improved hemodynamics and cerebral perfusion. The results of our experiment are to support the hypothesis that cerebral perfusion changes are reversible. The time required for this is between one and two weeks.

It was shown that rivastigmine at a dose of 1 mg/kg antagonized the impairing effect of IIAP in shuttle box active avoidance test [24]. The dose of 1 mg/kg rivastigmine was selected on the basis of data obtained from our previous experiments. The naïve rats treated with 1 mg/kg rivastigmine showed better results on learning and memory under various experimental methods compared to those treated with 2 mg/kg [28].

Rivastigmine also improves learning of rats with IIAP in step-through passive avoidance test and long-term memory in both passive avoidance tests of the same animals.

These effects support the data that rivastigmine affects oxidative damage and improves mithohondiral function in various brain regions - striatum, hippocampus and cortex [29]. There are experimental evidences for the beneficial effects of cholinomimetic agents such as rivastigmine and galatamine in improving memory outcomes following global cerebral ischemia reperfusion injury and the essential role of both muscarinic and nicotinic receptor functions to alleviate hippocampal neuronal death in CA1 region

[26]. Central cholinergic system is known to play an active role in learning and memory functions as well as a potent regulator of inflammatory immune responses [26, 30]. Global cerebral ischemia/reperfusion injury is characterized by substantial loss of cholinergic neurons, decreased brain acethylcholine levels and progressive decline of cholinergic functions in humans as well as experimental animals [31]. Therefore, cholinesterase inhibitors improve cognitive functions by enhancing acethylcholine levels in synapse. It was also found that brain cholinergic system has a unique attribute of including vasodilatation and association of improved cerebral blood flow and increased cognitive benefits [32, 33]. There are also substantial evidence indicating that rivastigmine improve cerebral blood flow [34].

CONCLUSION

Intra-abdomional hypertension and ACS are rare but potentially morbid diagnoses. Left untreated, ACS can lead to multisystem organ failure and death [35].

Study of increased intra-abdominal pressure and associated acute compartment syndrome as a cause of multisystem organ failure and the compounds for pharmacological responses are an important target for prevention and the optimal patient survival. Moreover, use of cholinesterase inhibitors such as rivastigmine will reduce the risk of cognitive impairment as a result of secondary cerebral hypoxia.

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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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