Effects of 3*R*,16*S*-2-hydroxyethyl apovincaminate (HEAPO), donepezil and galantamine on learning and memory retention in naïve Wistar rats

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ABSTRACT

The effects of 3*R*,16*S*-2-hydroxyethyl apovincaminate (HEAPO, RGH-10885) compared with those of two cholinesterase inhibitors, donepezil and galantamine, were examined in naïve Wistar rats using standard active and passive avoidance tests. The active avoidance test (shuttle box) and two passive avoidance tests (step-through and step-down) were performed according to the experimental design. There were 10 groups of rats (n = 8) and the substances studied were applied orally before each testing session. In the active avoidance test, the number of conditioned stimuli (avoidances), un-conditioned stimuli (escapes) and intertrial crossings were observed. In step-down and step-through passive avoidance tests, the latencies of reactions were observed. All the studied compounds showed positive

effects in the learning and memory tests, compared to the controls. It was concluded that HEAPO, donepezil and galantamine had a memory-enhancing effect in active and passive avoidance tests.

Keywords: vinpocetine derivatives, 3*R*,16*S*-2-hydroxyethyl apovincaminate (HEAPO), donepezil, galantamine, memory tests, rats

3*R*,16*S*-2-hydroxyethyl apovincaminate (HEAPO), a synthetic vinca alkaloid derivative, was studied. The main derivative of vinca alkaloids, vinpocetine (ethyl apovincaminate, EAPO) was discovered in the late 1960s and extracted from the leaves of the *Lesser periwinkle* (*Vinca minor*) (1). It is known that EAPO could be eliminated quickly from brain, while the elimination of HEAPO is slow and could remain for more than 12 h in the brain. Moreover, the contents of EAPO and HEAPO were found to be much higher in some brain structures, such as the hypothalamus, striatum and cortex than in the cerebellum (2). The latest *in vitro* studies have revealed the effect of EAPO on Ca^{2+}/calmodulin-dependent cyclic guanosine monophosphate phosphodiesterase 1, voltage-dependent Ca^{2+} channels, glutamate receptors and voltage-dependent Na^{+} channels (3). They suggest that the effect is relevant to the neuroprotective effect of EAPO. Some studies have tried to assess the efficacy and safety of EAPO in the treatment of patients with cognitive impairment due to vascular disease, Alzheimer's disease and other dementias (1).

Patients with Alzheimer's disease are reported to show an improvement in their cognitive function after treatment with acetylcholinesterase inhibitors such as donepezil over a 3- to 5-month period (4). There are also data indicating that cholinergic mechanisms may be, at least partially, responsible for hallucinations and delusions in patients with Parkinson's disease (5) as both of these effects improved significantly after 5 mg of donepezil for 2 months.

Donepezil is a potent and selective centrally acting reversible acethylcholinesterase (AChEI) inhibitor that has been proven to be effective in improving cognitive performance in patients with AD (6, 7). It can also attenuate the volume of cerebral infarction, protects against neuronal cell death and cognitive deficits following traumatic brain injury (8, 9) and enhances adult hippocampal neurogenesis (10). Recent studies have esta–blished the mechanisms by which donepezil modulates hippocampal neurogenesis (11) and the cholinergic anti-

inflammatory pathway (12). It has also been shown in rats that extracellular acetylcholine levels in the CNS increased after donepezil treatment (1.5 mg kg^{-1} *p.o.*) of ageing rats for 21 days (13). Higgins *et al*. (14) have shown that donepezil improves short-term memory in rats with scopolamine-induced amnesia.

Galantamine is an example of a plant alkaloid used in pharmacology (12). It was isolated from the bulbs and flowers of *Galanthus* species in the middle of the last century. Galantamine has been used as a competitive and reversible cholinesterase inhibitor and N-cholinergic allosteric modulator, having therapeutic significance for the treatment of peripheral paresis and for improving cholinergic deficits in the brain (15, 16). The behavioral studies in rodents indicated that galantamine improves hippocampal-dependent memory (17).

In contrast to clinical treatment by donepezil and galantamine, HEAPO is not clinically approved yet for treatment of Alzheimer's disease and other types of dementia (18). Some old data showed that EAPO, has cognitive activation ability in models of both scopolamine-induced and hypoxia-induced memory impairment in rats (19). The pharmacology studies continue to find new, chemically different compounds with memory-improving properties to treat dementia. Over the last decades, many studies on vincamine and its derivatives have confirmed their beneficial cerebrovascular effect, including neuroprotective activity. The combined results of *in vitro* and *in vivo* tests and the assessment of metabolism have identified 3*R*,16*S*-2'-hydroxyethyl apovincaminate (HEAPO,-RGH-10885) as the most promising compound, owing to its potent neuroprotective and antiamnesic activities (20).

The aim of the present study was to compare the effects of orally-administered HEAPO with those produced by donepezil and galantamine on learning and memory processing in naïve Wistar rats using active and passive avoidance tests.

EXPERIMENTAL

Chemicals

RGH-10885 [2-hydroxyethyl (3*R*,16*S*)-apovincaminate hydrochloride or 2-hydroxyethyl (41*S*,13a*R*)-13a-ethyl-2,3,41,5,6,13a-hexahydro-1*H*-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridine-12-carboxylate, abbreviated as HEAPO] and galantamine hydrobromide

were purchased from Gedeon Richter Ltd. (Hungary). Donepezil was obtained from pulverized Aricept 10-mg film coated tablets (Pfizer, USA): 1 tablet declared for 10 mg donepezil hydrochloride, namely, 9.12 mg donepezil free base. HEAPO, galantamine hydrobromide, and donepezil hydrochloride were dissolved just prior to use in 2 % hydroxypropyl methylcellulose solution (HPMC) at a concentration of 1 mg mL^{-1}. After appropriate diluting their final concentrations were $7.4 \times 10^{-3} – 7.4 \times 10^{-2}$ mol L^{-1}, $2.7 \times 10^{-4} – 2.7 \times 10^{-3}$ mol L^{-1}, and $2.4 \times 10^{-4} – 2.4 \times 10^{-3}$ mol L^{-1}, resp.

Doses of cholinesterase inhibitors were selected based on literature data on the effects of donepezil and galantamine on various behavioral tests for learning and memory in rodents (21, 22). Doses used for vinpocetine in experimental pharmacology were taken into account when selecting doses for RGH-10885 (23). Detailed data on the synthesis and chemical structure of RGH-10885 were provided by Nemes *et al*. (20).

Animals

The animals used in the experiments were male albino Wistar rats (3 months of age) with a body mass of 200–230 g. The total number of animals used in the experiments was 80, each group consisting of 8 animals. All experimental rats were housed on a 12-hour light/dark cycle under controlled temperature and lighting conditions, while food and water were provided *ad libitum*. The compounds in all experimental groups were applied per lavage (*per os*) as follows:

1st group (control group): 2 % HPMC, 0.1 mL per 100 g b.m.

2nd group: RGH-10885 3 mg kg^{-1} b.m.

3th group: RGH-10885 10 mg kg^{-1} b.m.

4th group: RGH-10885 30 mg kg^{-1} b.m.

5th group: donepezil hydrochloride 0.1 mg kg^{-1} b.m.

6th group: donepezil hydrochloride 0.5 mg kg^{-1} b.m.

7th group: donepezil hydrochloride 1.0 mg kg^{-1} b.m.

8th group: galantamine hydrobromide 0.1 mg kg^{-1} b.m.

9th group: galantamine hydrobromide 0.5 mg kg^{-1} b.m.

10th group: galantamine hydrobromide 1.0 mg kg^{-1} b.m.

The compounds were administered every day for 32 days, 30 minutes before the experiment (Table I).

All the experiments were conducted according to the requirements and regulations for working with laboratory animals in the EU (European Directive 2010/63/EU). Official permission for the study was obtained from the Ethical Committee of the Bulgarian Food Safety Agency and Protocol of the Ethics Committee at the Medical University Plovdiv. The animals were provided by the Animal House of Medical University-Plovdiv, Bulgaria.

Methods

The experimental methods applied have been used in our previous research on the learning and memory processes in rats (24–26). Drugs were administered daily throughout the test period.

Two-way active avoidance test (shuttle-box). – This test was performed in a standard shuttle box (Ugo Basile, Italy). The learning session consisted of a 5-day trial period using the standard program with 30 trials per day. In each trial 6 s light and buzzer (670 Hz, 70 dB), followed by 0.4 mA foot stimulation of 4 s duration and 12 s pause between shocks were applied. The parameters counted automatically were: (*i*) number of conditioned responses, *i.e*., avoidances; (*ii*) number of un-conditioned responses, *i.e*., escapes; (*iii*) number of intertrial crossings, and (*iv*) latency of reaction in seconds.

Memory retention test was made on day 12th, seven days after the last day of training, with same parameters for light and buzzer but with less electrical stimulation of the feet of 0.2 mA (see Table I).

Passive avoidance (step-through). – Step-through test was performed in an automatic set-up for passive avoidance (Ugo Basile), which consists of light and dark compartments. Each rat was placed in the light chamber. The door between chambers is closed for 6 s, followed by 12 s opened door which allows the rat entry into dark chamber. When the animal enters in the dark chamber the door closed automatically and the rat received a 0.4 mA foot shock for 9 s. Learning sessions were performed over two consecutive days, short memory test was made 24

hours later (3rd day) and a long memory retention session was performed on the 10th day. Memory retention test was conducted with the same parameters without foot shock. Sessions consisted of 3 trials separated by 30-minute interval. The learning criterion used was a latency of reactions for 180 s (3 min) staying in the light chamber in two consecutive trials.

In memory retrieval, a memory retention session of three trials per session were performed, 24 hours and 7 days after the learning sessions. Every trial consisted of the same parameters as above with a foot shock of 0.2 mA.

Passive avoidance test (step-down). – The rats were placed on a raised platform in the middle of the wire floor of a cage. The counter was then started and when the rat had placed at least three paws on the wire floor it received a foot shock. This was activated by pressing a button that delivered the stimulus for 10 s. A latency of 60 s in two consecutive trials was considered a task learned by the rat. Trials were performed in a ”step-down” apparatus (Ugo Basile) in a two-day learning session with 3 trials one hour apart, 0.4 mA foot shock and 0 frequency of shaking vibration of the platform (no shakes).

Twenty-four hours (short-term memory) and 7 days (long-term memory retrieval) after the learning session, a memory retention session of 3 trials each was performed. Every trial consisted of a foot shock of 0.2 mA and no shaking platform.

The same criterion for learning, *i.e.*, a latency of 60 s in two consecutive trials, was used before the rat was removed and assumed to have learned or memorized the task.

Statistical processing

Statistical analyses were performed on an INSTAT computer program using ANOVA for repeated measurements and the *post-hoc* Kruskall-Wallis test and Tukey-Kramer multiple comparison test. $p < 0.05$ was indicates a significant difference.

RESULTS AND DISCUSSION

Active avoidance test

In the active avoidance test the control rats produced an increased number of conditioned stimuli (avoidances) by days 4 and 5 of the learning session ($p < 0.05$) and on day 12 (memory retrieval test) ($p < 0.05$) compared to day 1 (Fig. 1). The rats treated with donepezil are illustrated in Fig. 1a. At a dose of 0.1 mg kg^{-1} donepezil showed an increased number of avoidances on day 5 of training ($p < 0.05$) compared to the controls at days 1 and 5, at a dose of 0.5 mg kg^{-1} it showed an increased number of avoidances during days 2–5 of the learning session ($p < 0.05$) compared to the controls (day 1 and respective days), and produced an increased number of avoidances in the memory retention test (12^{th} day) compared to the controls (day 1 and the same days), and at a dose of 1.0 mg kg^{-1} donepezil showed an increased number of conditioned responses (avoidances) on days 3 and 5 of the learning session ($p < 0.05$) compared to the day 1 control and the same days control, also showing an increased number of avoidances in the memory retention test (day 12) compared to the day 1 control and to the same days control.

RGH treatments are presented in Fig. 1b. The rats receiving 3 mg RGH-10885 kg^{-1} produced significantly more avoidances on days 4 and 5 of the learning session compared to the day 1 control and to the control of the corresponding day, and did not change it in the retention test (day 12) compared to the controls. The rats treated with RGH-10885 at a dose of 10 mg kg^{-1} showed an increased number of avoidances during 2^{nd} till 5^{th} day of learning ($p < 0.05$) compared to the day 1 control and to the controls of the corresponding days. An increased number of avoidances was recorded in the memory retention test (day 12) compared to the day 1 control as well as to the day 12 control ($p < 0.05$). However, at a dose of 30 mg kg^{-1} RGH-10885 showed no changes in the number of avoidances during the 5-day learning session or in the retention test (day 12), compared to the day 1 control or to the same day control.

Fig. 1c describes galantamine treatment. Galantamine, 0.1 mg kg^{-1}, produced an increased number of avoidances on days 3, 4 and 5 of training ($p < 0.05$), compared to the day 1 control as well as to the same days controls. The same group increased the number of avoidances on the 12^{th} day (memory retrieval test) compared to the 1^{st} day control ($p < 0.05$). The rats treated with galantamine, 0.5 mg kg^{-1}, showed no change in the number of conditioned responses (avoidances) during learning, or in the retention session compared to the day 1 controls or to the controls of the same days. Galantamine 1.0 mg kg^{-1} produced an increased number of conditioned stimuli on days 2, 3 and 5 of learning ($p < 0.05$), compared to the day 1

control or to the same day controls. An increased number of avoidances ($p < 0.05$) was recorded on day 4 of learning, compared to the day 1 control; in the memory retention test, galantamine produced a greater number of avoidances ($p < 0.05$) compared to the controls of day 1. In the active avoidance test, the control rats showed no significant change in the number of un-conditioned responses, *i.e.*, escapes during the 5-day learning or in the memory retention session, compared to the first day control (Table II). The control group rats produced a slightly fewer inter-trial crossings from day 2 to day 5 of the learning session and this tendency did not change in the memory retrieval test (Table III).

Also, the animals treated with donepezil, RGH-10885 or galantamine at three doses did not produce any significant changes in the number of un-conditioned stimuli, *i.e.*, escapes during the 5-days learning session, or in the memory retention test on day 12, compared to 1st day control (Table II). The same group did not exhibit any significant changes in the number of inter-trial crossings during the 5-day learning session or in the memory retention test on day 12, compared to the same day controls (Table III).

Passive avoidance tests

In the first passive avoidance test (step-through), the control rats spent almost the same time in the light chamber during learning and in the long-term memory retention tests, but time was longer on day 2 of learning and in the short-term memory retrieval test ($p < 0.05$), compared to the day 1 control (Fig. 2).

Donepezil at doses of 0.1 mg kg^{-1} and 1 mg kg^{-1} increased the latency of reaction on day 2 of the learning session, in the short-term memory retrieval or in the long-term memory retention tests ($p < 0.05$), compared to the day 1 control. However, at 0.5 mg kg^{-1} donepezil increased the latency of reaction on day 2 of the learning session and in the long-term memory test ($p < 0.05$) compared to the day 1 control and the same day control, and increased the latency of reactions in the short-term retrieval test ($p < 0.05$), compared to the day 1 control (Fig. 2a). RGH-10885, 3 mg kg^{-1}, increased the latency of reaction on day 2 of the learning session and in the short-term and long-term memory retention tests ($p < 0.05$), compared to the day 1 control and the same day control. RGH-10885, 10 mg kg^{-1} and 30 mg kg^{-1}, increased the latency of reaction on day 2 of the learning session ($p < 0.05$) compared to the day 1 control. It also

increased the latency of reaction in the short-term and long-term memory retrieval tests ($p < 0.05$) compared to the day 1 and the same day controls (Fig. 2b). Galantamine, 0.1 mg kg^{-1}, prolonged the latency of reaction in the short-term and long-term memory retention tests ($p < 0.05$) compared to the day 1 control. When treated with 0.5 mg kg^{-1} galantamine, prolonged latency of reaction on day 2 of the learning session or in the long-term memory retention tests ($p < 0.05$) compared to the day 1 control was seen. It also prolonged the latency of reaction in the short-term memory retention tests ($p < 0.05$) compared to the day 1 control as well as to the same day control. Galantamine, 1.0 mg kg^{-1}, also prolonged the latency of reaction on day 2 of the learning session ($p < 0.05$), compared to the day 1 control, and increased the latency of reaction in both memory tests ($p < 0.05$), compared to the day 1 control and the same day control group (Fig. 2c).

In the second passive avoidance test (step-down) the control rats spent almost the same time on the raised platform during the learning session, but increased latency ($p < 0.05$) in the short-term memory and long-term memory retention tests, compared to the day 1 controls (Fig. 3).

For rats treated with donepezil see Fig. 3a. Donepezil, 0.1 mg kg^{-1}, did not change their latency of reaction on the day 2 of the learning session, but increased it in the short-term memory retrieval test ($p < 0.05$) compared to the day 1 control and to the same day control, and in the long-term memory retention test ($p < 0.05$) compared to the day 1 control. At a dose of 0.5 mg kg^{-1} it did not change the latency of reaction on the day 2 of learning session, but increased it in the short-term or long-term memory retrieval tests ($p < 0.05$) compared to the day 1 control. Even at a higher dose of 1 mg kg^{-1} donepezil increased the latency of reaction on day 2 of the learning session ($p < 0.05$) compared to the day 1 control, as well as in the short-term memory retention test and in the long-term memory retrieval test ($p < 0.05$) compared to the day 1 control.

RGH-10885-treated rats are presented in Fig 3b. Rats receiving doses of 3, 10 or 30 mg kg^{-1} RGH-10885 did not change their latency of reaction (time spent on the platform was almost the same as for the controls) in the learning session, but increased it in the short-term, as well as in the long-term memory retention tests ($p < 0.05$), compared to the day 1 controls and to the same day controls.

Behavior of the galantamine-treated rats is displayed in Fig. 3c. Galantamine, 0.1 mg kg^{-1}, showed longer response latency on day 2 of the learning session and in the long-term memory retention tests ($p < 0.05$) than the controls of day 1 and the same day, and longer response latency in the short-term memory retention test ($p < 0.05$) when compared to the day 1 control. At a dose of 0.5 mg kg^{-1} galantamine also produced prolonged latency of reaction on day 2 of the learning session ($p < 0.05$) if compared to the day 1 control and the same day controls, and increased reaction latency was also recorded in the short-term or long-term memory retrieval tests ($p < 0.05$) compared to the day 1 control. At its highest dose, 1 mg kg^{-1}, galantamine produced latency of reaction ($p < 0.05$) on day 2 of the learning session and in the short-term memory retrieval test compared to the day 1 control and the same day controls. In the long-term memory retention test, it prolonged the latency of reactions ($p < 0.05$) compared to the day 1 control.

Summary

The essence of the active learning test is to increase the number of conditional responses of the control group during the training session and the memory test compared to the day 1 of the training. A behaviour experiment to study memory is valid only if the control group adequately learns the tasks (27). Moreover, all the parameters studied for the control group showed a tendency to increase during the experiment when compared to day 1, suggesting that the learning process had taking place. According to the aforementioned criteria, our active avoidance experiment is valid, because the control group showed a significant increase in the number of avoidances over the training period and in the memory retention test.

It is interesting that, like donepezil, RGH-10885 produced better enhancing effects at the medium dose and showed a bell-shaped dose-response effect. Galantamine also displayed some enhancing effect, better expressed at the lowest and highest doses, but the dose-response is inverted bell-shaped curve.

The mechanism by which the entire class of vincamine-type compounds, to which RGH-10885 belongs, enhances cognition is not fully established. They are classified as nootropics (28). Nootropics improve learning and memory through stimulation of cholinergic neurotransmission by inhibiton of enzyme acethylcholinesterase, uptake of choline, positive allosteric modulation for acetylcholine and glutamate receptor, enhance the release of dopamine

(29). Vincamine is known to act as a ligand and allosteric modulator of M_1 to M_4 receptors in the cerebral cortex and hippocampus (20, 30).

The acetylcholine in the CNS exerts numerous functions, because during spatial acquisition learning acetylcholine efflux into the extracellular space in hippocampus and cortex increases, but during consolidation of reference memory acetylcholine levels are low. This explains why acetylcholine receptor blockade during acquisition blocks memory formation and it is consonant with the notion that an unspecific enhancement of cholinergic activity during consolidation is determinant to memory formation (31).

Donepezil is the most-prescribed medication in Alzheimer's disease because patients tolerate it well and it has a good safety profile. Recent clinical trials have confirmed its efficacy and safety when given at the usual doses of 5 mg or 10 mg daily in patients with mild to moderate Alzheimer's disease (32). Donepezil has beneficial effects at the cellular and molecular acetylcholine levels, which has been demonstrated *in vitro* and in animal studies (33, 34). Recent studies explain the good therapeutic results of donepezil in Lewy body dementia with stimulation of M_1-M_4 muscarinic receptors (35). As we mentioned above, RGH-10885 and donepezil show the best cognitive function-enhancing effect when administered in a medium dose. It could be hypothesized that this is due to the ability of both substances to activate the same muscarinic receptors in the brain. In contrast, in our experiments galantamine also produced a learning and memory-enhancing effect when used in low and higher doses. Its mechanism of action includes acetylcholinesterase inhibition and modulation of brain alpha-7 nicotinic receptors (36).

CONCLUSIONS

In active avoidance test (shuttle-box), RGH-10885 (3*R*,16*S*-2'-hydroxyethyl apovincaminate), in a medium dose, improved learning process and long-term memory and this effect was comparable with the effects of the reference drug donepezil. The second reference drug galantamine had a weak effect on learning behaviour and had no effect on memory retention.

In step-through passive avoidance test the all tested doses of RGH-10885 improved learning and enhanced the short- and long-term memory similarly to the donepezil.

In step-down passive avoidance test RGH-10885 did not affect acquisition but had improving effect on memory consolidation. Short-term memory is best affected by the highest dose of RGH-10885 and galantamine, and long-term by the medium dose of RGH-10885.

It can be concluded that RGH-10885 has an improving effect on the learning and memory processes in naïve rats in both active and passive avoidance tests. The exact mechanisms of the effect of RGH-10885 need to be further examined.

Our future plans involve study the effects of RGH-10885 on female rats and determine the sex differences, and on animals with experimental models of impaired memory as well as toxicological studies.

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REFERENCES

1. S. Z. Szatmári and P. J. Whitehouse, Vinpocetine for cognitive impairment and dementia, *Cochrane Database Syst. Rev*. **1** (2003) CD003119; https://doi.org/10.1002/14651858.CD003119

2. M. Wang, L. Wang, J. Sun, L. Zhang, L. Zhao and X. Xiong, Simultaneous determination of vinpocetine and its major active metabolite apovincaminic acid in rats by UPLC-MS/MS and its application to the brain tissue distribution, *J. Chromatogr. Sci*. **56**(3) (2018) 225–232; https://doi.org/10.1093/chromsci/bmx104

3. P. Bönöczk, B. Gulyás, V. Adam-Vizi, A. Nemes, E. Kárpáti, B. Kiss, M. Kapás, C. Szántay, I. Koncz, T. Zelles and A. Vas, Role of sodium channel inhibition in neuroprotection: effect of vinpocetine, *Brain Res. Bull*. **53**(3) (2000) 245–254; https://doi.org/10.1016/S0361-9230(00)00354-3

4. I. T. Lott, K. Osann, E. Doran and L. Nelson, Down syndrome and Alzheimer disease – Response to donepezil, *Arch. Neurol*. **59**(7) (2002) 1133-1136; https://doi.org/10.1001/archneur.59.7.1133

5. G. Fabbrini, P. Barbanti, C. Aurilia, C. Pauletti, G. L. Lenzi and G. Meco, Donepezil in the treatment of hallucinations and delusions in Parkinson's disease, *Neurol. Sci*. **23**(1) (2002) 41–43; https://doi.org/10.1007/s100720200022

6. H. Ogura, T. Kosasa, Y. Kuriya and Y. Yamanishi, Donepezil, a centrally acting acethylcholinesterase inhibitor, alleviates learning deficits in hypocholinergic models in rats, *Methods Find. Exp. Clin. Pharmacol*. **22**(2) (2000) 89–95; https://doi.org/10.1358/mf.2000.22.2.796070

7. D. R. Liston, J. A. Nielsen, A. Villalobos, D. Chapin, S. B. Jones, S. T. Hubbard, I. A. Shalaby, A. Ramirez, D. Nason and W. F. White, Pharmacology of selective acethylcholinesterase inhibitors: implications for use in Alzheimer's disease, *Eur. J. Pharmacol*. **486**(1) (2004) 9–17; https://doi.org/10.1016/j.ejphar.2003.11.080

8. A. Khateb, J. Amman, J. M. Annoni and K. Diserens, Cognition-enhancing effects of donepezil in traumatic brain injury, *Eur. Neurol*. **54**(1) (2005) 39–45; https://doi.org/10.1159/000087718

9. M. Fujiki, H. Kobayashi, S. Uchida, R. Inoue and K. Ishii, Neuroprotective effect of donepezil, a nicotinic acethylcholine-receptor activator, on cerebral infarction in rats, *Brain Res*. **1043**(1-2) (2005) 236–241; https://doi.org/10.1016/j.brainres.2005.02.063

10. S. Kotani, T. Yamauchi, T. Teramoto and H. Ogura, Donepezil, an acethylcholinesterase inhibitor, enhances adult hippocampal neurogenesis, *Chem. Biol. Interact* **175**(1-3) (2008) 227–230; https://doi.org/10.1016/j.cbi.2008.04.004

11. K. J. Kwon, M. K. Kim, E. J. Lee, J. N. Kim, B. R. Choi, S. Y. Kim, K. S. Cho, J. S. Han, H. Y. Kim, C. Y. Shin and S. H. Han, Effects of donepezil, an acetylcholinesterase inhibitor, on neurogenesis in a rat model of vascular dementia, *J. Neurol. Sci*. **347**(1-2) (2014) 66–77; https://doi.org/10.1016/j.jns.2014.09.021

12. M. Pohanka, Inhibitors of acethylcholinesterase and butyrylcholinesterase meet immunity, *Int. J. Mol. Sci.* **15**(6) (2014) 9809–9825; https://doi.org/10.3390/ijms15069809

13. C. Scali, F. Casamenti, A. Bellucci, C. Costagli, B. Schmidt and G. Pepeu, Effect of subchronic administration of metrifonate, rivastigmine and donepezil on brain acetylcholine in aged F344 rats, *J. Neural Transm*. **109** (2002) 1067–1080; https://doi.org/10.1007/s007020200090

14. G. A. Higgins, M. Enderlin, R. Fimbel, M. Haman, A. J. Grottick, M. Soriano, J. G. Richards, J. A. Kemp and R. Gill, Donepezil reverse a mnemonic deficit produced by scopolamine but not by perforant path lesion or transient cerebral ischemia, *Eur. J. Neurosci*. **15**(11) (2002) 1827–1840; https://doi.org/10.1046/j.1460-9568.2002.02018.x

15. W. J. Krall, J. J. Sramek and N. R. Cutler, Cholinesterase inhibitors: a therapeutic strategy for Alzheimer disease, *Ann. Pharmacother*. **33**(4) (1999) 441–450; https://doi.org/10.1345/aph.18211

16. A. Nordberg and A.-L. Svenson, Cholinesterase inhibitors in the treatment of Alzheimer's disease - A comparison of tolerability and pharmacology, *Drug Safety* **19** (1998) 465–480; https://doi.org/10.2165/00002018-199819060-00004

17. C. Yuede, H. Dong and J. G. Csernansky, Anti-dementia drugs and hippocampal-dependent memory in rodents, *Behav. Pharmacol*. **18**(5-6) (2007) 347–363; https://doi.org/10.1097/FBP.0b013e3282da278d

18. M. J. H. J. Dekker, J. C. Bouvy, D. O'Rourke, R. Thompson, A. Makady, P. Jonsson and C. C. Gispen-de Wied, Alignment of European regulatory and health technology assessments: A review of licensed products for Alzheimer's disease, *Front. Med*. (Lausanne) **6** (2019) Article ID 73 (9 pages); https://doi:10.3389/fmed.2019.00073

19. V. J. DeNoble, S. J. Repetti, L. W. Gelpke, M. Wood and K. L. Keim, Vinpocetine: nootropic effects on scopolamine-induced and hypoxa-induced retrieval deficits of a step-through passive avoidance response in rats, *Pharmacol. Biochem. Behav*. **24**(4) (1986) 1123–1128; https://doi.org/10.1016/0091-3057(86)90465-X

20. A. Nemes, L. Czibula, C. Szántay, A. Gere, B. Kiss, J. Laszy, I. Gyertyán, Z. Szombathelyi and C. Szántay, Synthesis and evaluation of 2'-hydroxyethyl trans-apovincaminate derivatives as antioxidant and cognitive enhancer agents, *J. Med. Chem*. **51**(3) (2008) 479–486; https://doi.org/10.1021/jm070618k

21. J. Prickaerts, A. Sick, F. J. van der Staay, J. de Vente and A. Blokland, Dissociable effects of acethylcholinesterase inhibitors and phosphodiesterase type 5 inhibitors on object recognition memory: acquisition versus consolidation, *Psychopharmacology* **177** (2005) 381–390; https://doi.org/10.1007/s00213-004-1967-7

22. F. Jia, M. Kato, H. Dai, A. Xu, T. Okuda, E. Sakurai, N. Okamura, T. W. Lovenberg, A. Barbier, N. I. Carruthers, K. Linuma and K. Yanai, Effects of histamine H3 antagonists and donepezil on learning and mnemonic deficits induced by pentylentetrazol kindling in weanling mice, *Neuropharmacology* **50**(4) (2006) 404–411; https://doi.org/10.1016/j.neuropharm.2005.09.017

23. V. J. DeNoble, Vinpocetine enhances retrieval of a step-through passive avoidance response in rats, *Pharmacol. Biochem. Behav.* **26**(1) (1987) 183–186; https://doi.org/10.1016/0091-3057(87)90552-1

24. D. Dimitrova and D. Getova-Spassova, Effects of galantamine and donepezil on active and passive avoidance tests in rats with induced hypoxia, *Pharmacol. Sci.* **101** (2006) 199–204; https://doi.org/10.1254/jphs.fpe05006x

25. D. P. Getova and D. D. Dimitrova, Effects of $GABA_B$ receptor antagonists CGP63360, CGP76290A and CGP76291A on learning and memory processes in rats, *Centr. Eur. J. Med.* **2**(3) (2007) 280–293; https://doi.org/10.2478/s11536-007-0033-3

26. L. V. Vasileva, D. P. Getova, N. D. Doncheva, A. S. Marchev and M. I. Georgiev, Beneficial effect of commercial *Rhodiola* extract in rats with scopolamine-induced memory impairment on active avoidance, *J. Ethnopharmacol.* **193** (2016) 586–591; https://doi.org/10.1016/j.jep.2016.10.011

27. J. A. Quillfeldt, *Behavioral Methods to Study Learning and Memory in Rats*, in *Rodent Model as Tools in Ethical Biomedical Research* (Eds. M. L. Andersen and S. Tufik), Springer Cham, Heidelberg 2016, pp. 101–136.

28. W. Froestl, A. Muhs, A. Pfeifer, Cognitive enhancers (nootropics). Part 1: drugs interacting with receptors, *J. Alzheimers Dis*. **32**(4) (2012) 793–887; https://doi.org/10.3233/JAD-2012-121186

29. N. A. Suliman, C. N. Mat Taib, M. A. Mohd Moklas, M. I. Adenan, M. T. Hidayat Baharuldin, R. Basir, Establishing natural nootropics: Recent molecular enhancement influenced by natural nootropic, *Evid. Based Complement. Alternat. Med*. **2016** (2016) Article ID 4391375 (12 pages); https://doi.org/10.1155/2016/4391375

30. J. Jakubík, L. Bačáková, E. E. El-Fakahany and S. Tuček, Positive cooperativity of acetylcholine and other agonists with allosteric ligands on muscarinic acetylcholine receptors, *Mol. Pharmacol*. **52**(1) (1997) 172–179; https://doi.org/https://doi.org/10.1124/mol.52.1.172

31. S. Deiana, B. Platt and G. Riedel, The cholinergic system and spatial learning, *Behav. Brain Res*. **221**(2) (2011) 389–411; https://doi.org/10.1016/j.bbr.2010.11.036

32. J. Jia, C. Wei, W. Chen, L. Jia, A. Zhow, F. Wang, Y. Tang and L. Xu, Safety and efficacy of donepezil 10 mg/day in patients with mild to moderate Alzheimer's disease, *J. Alzheimers Dis.* **74**(1) (2020) 199–211; https://doi.org/10.3233/JAD-190940

33. S. A. Jacobson and M. N. Sabbagh, Donepezil: potential neuroprotective and disease-modifying effects, *Expert Opin. Drug Metab. Toxicol*. **4**(10) (2008) 1363–1369; https://doi.org/10.1517/17425255.4.10.1363

34. H. G. Kim, M. Moon, J. G. Choi, G. Park, A.-J. Kim, J. Hur, K.-T. Lee and M. S. Oh, Donepezil inhibits the amyloid-beta oligomer-induced microglial activation *in vitro* and *in vivo*, *Neurotoxicology* **40** (2014) 23–32; https://doi.org/10.1016/j.neuro.2013.10.004

35. S. J. Colloby, P. J. Nathan, I. G. McKeith, G. Bakker, J. T. O'Brien, J.-P. Taylor, Cholinergic muscarinic M1/M4 receptor networks in dementia with Lewy bodies, *Brain Commun*. **2**(2) (2020) Article ID fcaa098 (12 pages); https://doi.org/10.1093/braincomms/fcaa098

36. S. A. Wazea, W. Wadie, A. K. Bahgat, H. S. El-Abhar, Galantamine anti-colitic effect: Role of alpha-7 nicotinic acetylcholine receptor in modulating Jak/STAT3, NF-κB/HMGB1/RAGE and *p*-AKT/Bcl-2 pathways. *Sci. Rep.* **8** (2018) Article ID 5110 (10 pages); https://doi.org/10.1038/s41598-018-23359-6

454 *Table I. Experimental design*

Day	1st week	2nd week	3rd week	4th week	5th week
1	Shuttle-box test	Only treatment	Step-through test Short-term memory retention	Step-through retest Long-term memory retention	Only treatment
2	Shuttle-box test	Only treatment	Only treatment	Step-down test	Only treatment
3	Shuttle-box test	Only treatment	Only treatment	Step-down test	Only treatment
4	Shuttle-box test	Only treatment	Only treatment	Step-down test Short-term memory retention	Step-down retest Long-term memory retention
5	Shuttle-box test	Shuttle-box retest long-term memory retention	Only treatment	Only treatment	
6	Only treatment	Step-through test	Only treatment	Only treatment	
7	Only treatment	Step-through test	Only treatment	Only treatment	

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Table II. Effects of donepezil, RGH-10885 and galantamine on the number of un-conditioned responses (escapes) in active avoidance test (shuttle box)

Drug and dose (mg kg^{-1} bm)[a]		Day of testing (mean ± SEM)[b]					
		1	2	3	4	5	12
Control		10.0 ± 2.0	10.0 ± 2.0	11.0±2.5	8.4 ± 1.6	9.6 ± 1.7	9.0 ± 1.5
Donepezil	0.1	14.7 ± 0.9	13.3 ± 0.8	15.0 ± 1.3	18.0 ± 1.7	16.0 ± 1.9	17.0 ± 1.6
	0.5	17.4 ± 2.1	19.2 ± 2.5	17.2 ± 1.8	12.7 ± 1.9	15.0 ± 1.6	16.3 ± 2.0
	1.0	17.2 ± 2.0	21.7 ± 2.6	20.3 ± 2.4	19.0 ± 3.0	12.3 ± 1.9	15.7 ± 1.9
RGH-10885	3	16.7± 1.5	16.0 ± 1.8	15.2 ± 1.7	15.7 ± 1.9	19.0 ± 2.2	17.3 ± 2.0
	10	24.7 ± 3.0	22.3 ± 3.2	21.7 ± 3.5	17.3 ± 2.2	15.8 ± 1.9	13.3 ± 1.9
	30	14.0 ± 1.7	12.7 ± 1.5	15.0 ± 1.9	11.7 ± 2.2	12.0 ± 2.5	13.6 ± 2.5
Galactamine	0.1	21.0 ± 3.5	20.5 ± 4.1	15.3 ± 2.4	20.7 ± 2.7	19.3 ± 2.1	21.7 ± 2.6
	0.5	15.3 ± 2.2	10.7 ± 1.8	10.4 ± 2.1	15.0 ± 2.7	15.7 ± 3.2	16.0 ± 2.9
	1.0	22.0 ± 3.7	23.5 ± 3.4	20.0 ± 3.8	22.6 ± 3.9	21.7 ± 2.9	22.9 ± 3.3

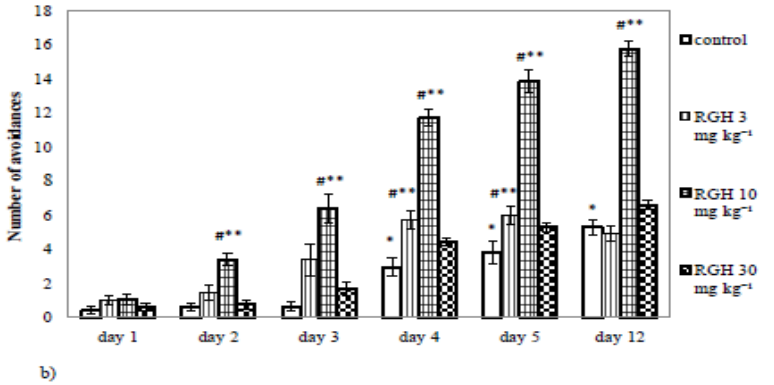
[a] Administered *per os*.
[b] n = 8 rats per group.

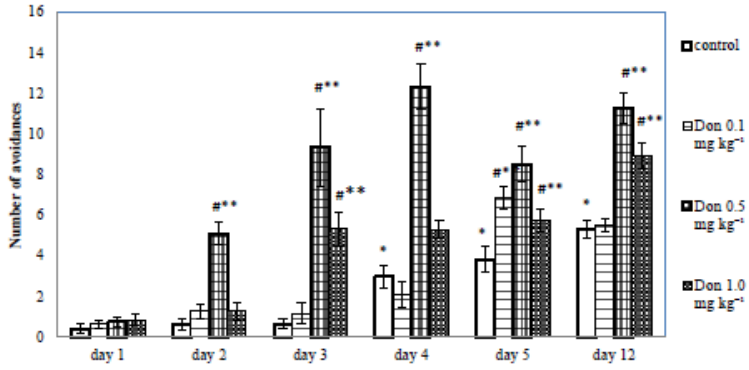
Table III. Effects of donepezil, RGH-10885 and galantamine on the number of inter-trial crossings in active avoidance test (shuttle box)

Drug and dose (mg kg^{-1} bm)[a]		Day of testing (mean ± SEM)[b]					
		1	2	3	4	5	12
Control		1.2 ± 0.4	0.4 ± 0.2	0.6 ± 0.4	1.1 ± 0.5	0.6 ± 0.3	0.8 ± 0.3
Donepezil	0.1	3.2 ± 0.6	2.5 ± 0.4	1.3 ± 0.4	2.2 ± 0.5	2 ± 0.8	1.2 ± 0.3
	0.5	2.5 ± 0.6	2.6 ± 0.5	2.3 ± 0.5	2.9 ± 0.6	2.5 ± 0.5	1.7 ± 0.4
	1.0	3.3 ± 0.7	2.5 ± 0.7	4.4 ± 1.0	3.3 ± 0.7	1.5 ± 0.5	2.3 ± 0.6
RGH-10885	3	2.5 ± 0.5	1.3 ± 0.2	1.4 ± 0.4	2.3 ± 0.7	2.2 ± 0.4	1.3 ± 0.4
	10	5.4 ± 0.9	2.4 ± 0.5	2.2 ± 0.3	2.3 ± 0.5	1.8 ± 0.2	2.2 ± 0.4
	30	3.8 ± 0.7	2.0 ± 0.4	2.3 ± 0.6	1.7 ± 0.3	2.3 ± 0.5	3.1 ± 0.6
Galactamine	0.1	1.7 ± 0.4	1.1 ± 0.3	2.6 ± 0.6	2.0 ± 0.2	2.1 ± 0.4	1.9 ± 0.3
	0.5	1.6 ± 0.3	1.0 ± 0.2	1.1 ± 0.2	1.6 ± 0.7	1.1 ± 0.5	1.3 ± 0.4
	1.0	2.5 ± 0.8	3.2 ± 0.7	2.3 ± 0.5	1.8 ± 0.5	1.9 ± 0.3	1.2 ± 0.4

[a] Administered *per os.*

[b] n = 8 rats per group.





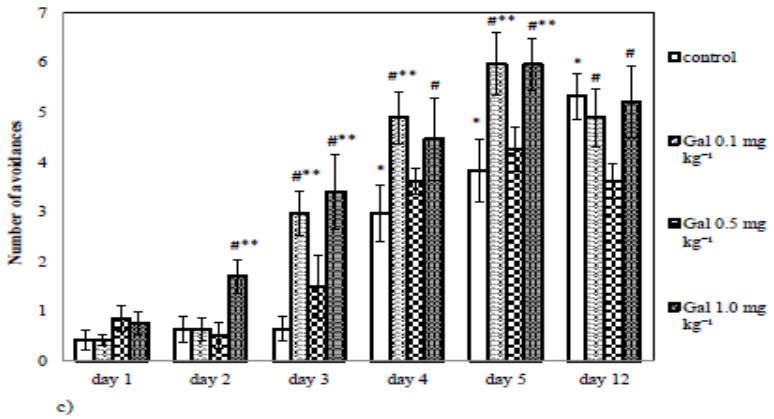
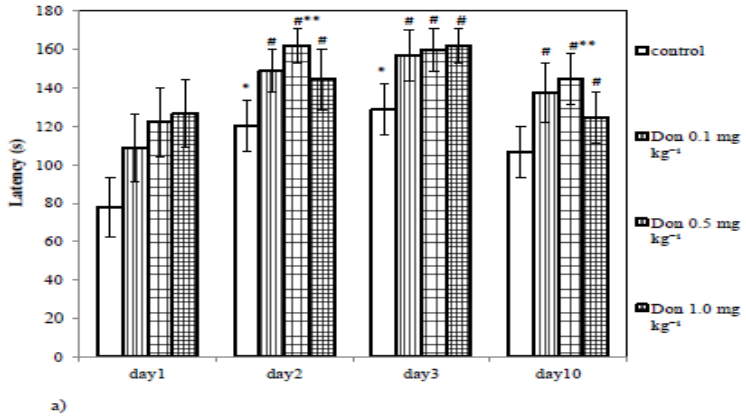
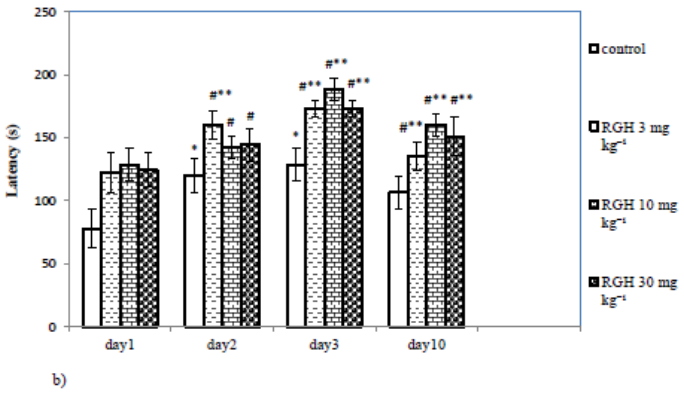


Fig. 1. Effects on conditioned responses (avoidances) in active avoidance test (shuttle box): a) donepezil, b) RGH-10885 c) galantamine. Significant difference *versus* control: $^{*}p < 0.05$ day 1 control, $^{**}p < 0.05$ same day control, $^{\#}p < 0.05$ day 1 control (Don – donepezil, RGH – RGH-10885, Gal –- galantamine).



a)



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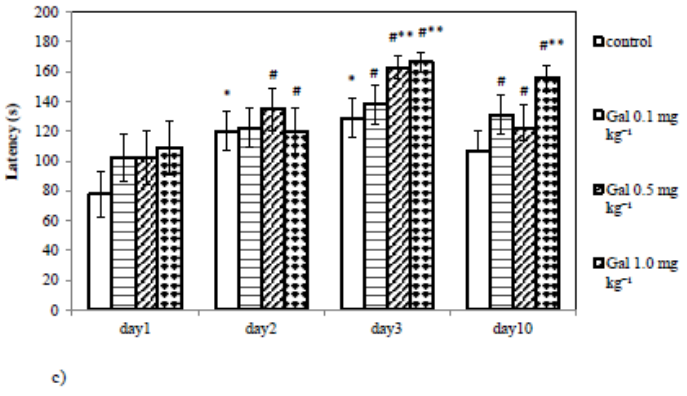
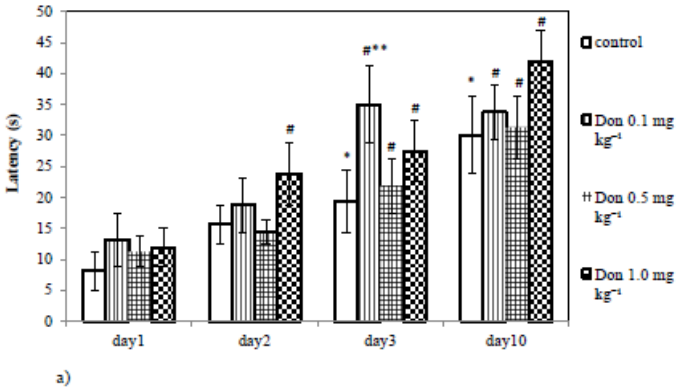


c)

Fig. 2. Effects on latency in step-through passive avoidance test: a) donepezil, b) RGH-10885, c) galantamine. Significant difference *versus* control: $^{*}p < 0.05$ day 1 control, $^{**}p < 0.05$ same day control, $^{\#}p < 0.05$ day 1 control (Don – donepezil, RGH – RGH-10885, Gal – galantamine).



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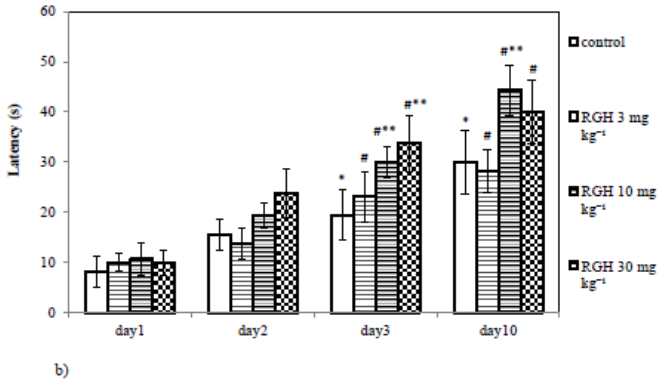

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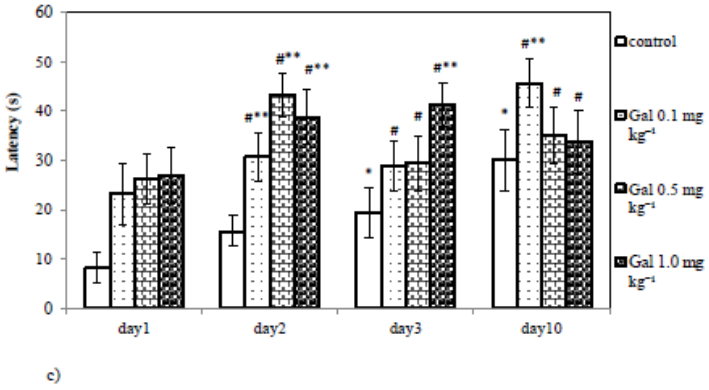

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487 Fig. 3. Effects on latency in step-down passive avoidance test: a) donepezil, b) RGH-10885, c)
488 galantamine. Significant difference *versus* control: $^{*}p < 0.05$ day 1 control; $^{**}p < 0.05$ same day control,
489 $^{\#}p < 0.05$ day 1 control (Don – donepezil, RGH – RGH-10885, Gal – galantamine).