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*Full Paper*

# Effects of Galantamine and Donepezil on Active and Passive Avoidance Tests in Rats With Induced Hypoxia

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**Abstract.** The cholinergic system undergoes changes with aging and in Alzheimer's disease. The effects of the anticholinesterase drugs galantamine and donepezil were studied in a model with sodium nitrite-induced hypoxia in rats. The animals were trained in the shuttle-box active avoidance test and in step-through and step-down passive avoidance tests. In the active avoidance test, hypoxic rats showed a decrease in the number of avoidances in the learning session and in retention. The hypoxic rats receiving galantamine showed an increase in the number of avoidances during the learning session. The groups in hypoxia treated with donepezil had an increased number of avoidances in the learning session. In memory retention tests, significant differences were not observed in the hypoxic animals treated with galantamine or donepezil. In the step-through passive avoidance test, rats treated with galantamine had no change in the latency of reactions during the learning session and memory retention tests. In the step-down passive avoidance test, the animals treated with galantamine had increase latency of reactions during the learning and short- or long-memory retention tests. The hypoxic rats receiving donepezil had increased latency of reactions in the step-down short memory retention test. Our results suggest that galantamine and donepezil improve cognitive functions in a model of hypoxia.

***Keywords***: sodium nitrite-induced hypoxia, cholinesterase inhibitor, galantamine, donepezil

## Introduction

The function of the cholinergic system is known to change during normal aging and in pathological conditions such as Alzheimer's disease (AD) (1, 2). The rationale for the development of cholinesterase inhibitors was the now-well known "cholinergic" hypothesis (1) that the progressive loss of cholinergic neurons seen in the AD brain and the resulting decline in levels of the neurotransmitter acetylcholine correlates with cognitive decline. The cholinesterase inhibitors are widely recommended for the treatment of mild to moderate AD (3).

Being a reversible inhibitor of the acetylcholinesterase, galantamine acts as an allosterically potentiating ligand on nicotine acetylcholine receptors ($\alpha 4/\beta 2$ subtype), making them more sensitive (4, 5). Iliev et al. (6) have observed a beneficial effect of galantamine on the recovery of learning ability after single administration in post-ischemic rats using the shuttle-box test. They proved that galantamine improves learning ability, short memory retention, and spatial orientation and suggest a direct effect on the early pathologenetic mechanisms of central nervous system (CNS) damage.

Higgins et al., (7) showed that the effect of donepezil on cognitive impairment by scopolamine was fully reversed in a test of short-term memory. Others (8) indicate that donepezil administered orally has a potent and selective effect on the central cholinergic system, increasing significantly and dose-dependently the extracellular acetylcholine concentration in the rat cerebral cortex.

Chronic application of sodium nitrite induces long

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lasting effects as hypoxia, neuron damage and impaired behavior (9). Using sodium nitrite hypoxia, a model of aging brain, Bhattacharya (10) studied the effects of an herbal formulation (Mentat) on learning acquisition in the elevated plus maze and step-down test and showed a facilitating effect.

The aim of our study was to analyze the effects of the anticholinesterase drugs galantamine and donepezil in a model of aging brain (sodium nitrite-induced hypoxia) using active and passive avoidance tests in rats.

## Materials and Methods

*Drugs*

Donepezil (Aricept; Pfizer, New York, NY, USA) is 2-3-dihydro-5,6-dimethoxy-2[[(1-phenylmethyl)-4-piperidiny]methyl]-1K-inden-1-one or (+/−)-2-[(1-benzyl-4-piperidyl) methyl]-5,6-dimethoxy-1-indamine.

Galantamine (Nivalin; Sopharma, Sofia, Bulgaria) is (4a*S*,6*R*,8a*S*)-4a,5,9,10,11,12-hexahydro-3-methoxy-11-methyl-6*H*-benzofuro-[3*a*,3,2-*ef*][2][benzozepin-6-ol.

*Animals*

Male Wistar rats weighing 180 – 220 g kept under standard laboratory conditions (08.00 – 20.00 light, food and water at libitum) were used. The animals were divided into the following experimental groups (n = 8): A: saline, s.c. + saline, p.o. (0.1 ml/100 g body weight); B: sodium nitrite, 50 mg/kg, s.c. + saline, p.o.; C: sodium nitrite, 50 mg/kg, s.c. + donepezil, 0.5 mg/kg, p.o.; D: sodium nitrite, 50 mg/kg, s.c. + donepezil, 1.0 mg/kg, p.o; E: sodium nitrite, 50 mg/kg, s.c. + galantamine, 0.5 mg/kg, p.o.; F: sodium nitrite, 50 mg/kg, s.c. + galantamine, 1.0 mg/kg, p.o. Immediately after the subcutaneous injection, the rats received per gavage the second application and the tests were performed 60 min later.

*Behavior tests*

An automatic reflex conditioner for active avoidance "shuttle box" (Ugo Basile, Comerio-Varese, Italy) was used. A learning session of 5 consecutive days was performed. Each day consisted of 30 trials with the following parameters: 6 s light and buzzer (670 Hz and 70 dB), 3 s 0.4-mA foot shock, 12 s pause. A memory retention session with the same parameters without foot shock was performed 7 days later (12th day). The parameters automatically counted were as follows: 1, number of conditioned responses (avoidances); 2, number of unconditioned stimuli (escapes from foot shock); 3, number of intertrial crossings.

Two passive avoidance tests were used:

Automatic set-up for passive avoidance "step-through" (Ugo Basile) was used. The test parameters were as follows: door delay −6 s, open door for 12 s, 0.4-mA foot shock 9 s later. A learning session of 2 consecutive days was performed; and 24-h later (3rd day), short memory retention session, and on the 10th day a long memory retention session were done. Each session consisted of 3 trials, and a 30-min pause between each. Learning criterion was latency of reactions of 180 ± 2 s in the light chamber.

An automatic set-up for passive avoidance "step-down" wire cage with plastic platform (Ugo Basile) was used. The learning session consisted of 2 trials (electrical stimulation duration of 10 s at intensity of 0.4 mA) with 60-min interval between trials. The learning session of 2 consecutive days was done; and 24-h later (3rd day), short memory retention session and on the 7th day a long memory retention session were made. The memory retention test with the same parameters without foot shock was done. The latency of reactions (the rat remaining on the platform for more than 60 s) was accepted as criterion for learning and retention.

*Statistical evaluation*

The data were analyzed with one-way ANOVA by the INSTAT computer program. The mean ± S.E.M. for each group was calculated. Two-way ANOVA for repeated measurements was used to compare the experimental groups with the corresponding control groups.

## Results

In the shuttle-box active avoidance test, the control rats showed a statistically significant increase in the number of avoidances on the 3rd ($P<0.05$), 4th, and 5th days ($P<0.01$) of the learning session compared to the 1st day and kept it in the memory retention session on the 12th day (Fig. 1).

The rats treated with sodium nitrite only showed a significant decrease in the number of avoidances on the 3rd, 4th, and 5th days of the learning session ($P<0.01$) and in the retention session ($P<0.05$) compared to the same day control group. The group treated with sodium nitrite and 0.5 mg/kg donepezil showed a significant increase in the number of avoidances ($P<0.05$) on the 3rd and 4th day of the learning session compared to the same day sodium nitrite group. The rats treated with sodium nitrite and higher dose donepezil (1.0 mg/kg) had significantly increased number of conditioned stimuli responses ($P<0.05$) on the 4th and 5th day of the learning session compared to the same day animals treated with sodium nitrite (Fig. 1). The animals in hypoxia and receiving smaller dose of galantamine had

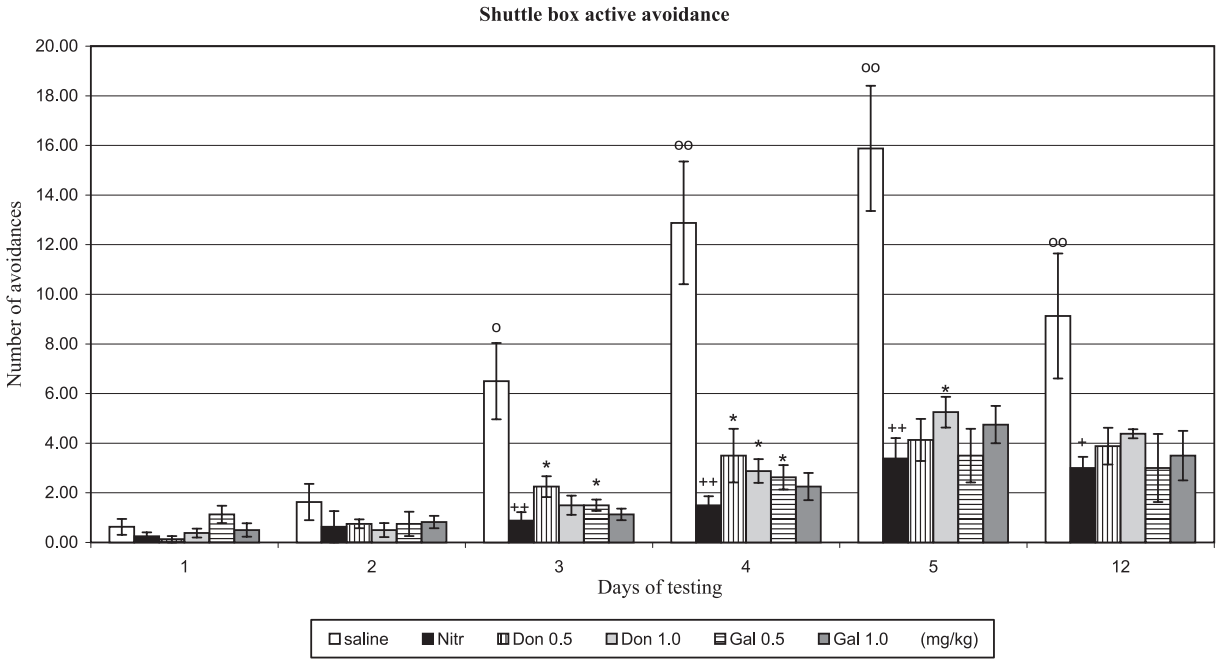


**Fig. 1.** Effects of galantamine (Gal) and donepezil (Don) on the number of conditioned stimuli responses (avoidances) in active avoidance test. On the abscissa – days of testing; on the ordinate – number of avoidances. $^{o}P<0.05$ and $^{oo}P<0.01$, compared to the 1st day control; $^{+}P<0.05$ and $^{++}P<0.01$, compared to the same day control; $*P<0.05$, compared to the same day of animals treated with sodium nitrite only.

**Table 1.** Effects of galanthamine and donepezil on active avoidance test (shuttle box) in a model of hypoxia: number of unconditioned stimuli responses (escapes)

| Days | Control Saline 0.1 ml/100 g | $NaNO_2$ 50 mg/kg + Saline 0.1 ml/100 g | $NaNO_2$ 50 mg/kg + donepezil 0.5 mg/kg | $NaNO_2$ 50 mg/kg + donepezil 1.0 mg/kg | $NaNO_2$ 50 mg/kg + galanthamine 0.5 mg/kg | $NaNO_2$ 50 mg/kg + galanthamine 1.0 mg/kg |
|---|---|---|---|---|---|---|
| 1 | 14.75 ± 1.95 | 6.88 ± 2.11$^{+}$ | 7.25 ± 1.88 | 8.13 ± 1.56 | 20.75 ± 2.76** | 12.00 ± 3.55* |
| 2 | 15.63 ± 2.41 | 5.75 ± 2.34$^{+}$ | 10.25 ± 2.82 | 8.00 ± 3.24 | 12.38 ± 3.02* | 6.63 ± 2.98 |
| 3 | 16.13 ± 2.52 | 8.63 ± 2.35$^{+}$ | 15.13 ± 2.98 | 9.63 ± 4.00 | 11.50 ± 3.37 | 8.00 ± 3.72 |
| 4 | 11.75 ± 2.23 | 6.88 ± 2.92 | 11.75 ± 2.88 | 11.38 ± 3.59 | 11.00 ± 3.74 | 9.13 ± 3.57 |
| 5 | 12.25 ± 1.90 | 8.00 ± 3.32 | 10.50 ± 2.99 | 8.75 ± 3.82 | 12.13 ± 2.81 | 8.88 ± 3.04 |
| 12 | 15.50 ± 3.17 | 8.25 ± 3.30 | 7.25 ± 2.34 | 8.75 ± 3.90 | 10.88 ± 2.92 | 5.75 ± 3.33 |

$^{+}P<0.05$, compared to the same day control; $*P<0.05$ and $**P<0.01$, compared to the same day of the group treated with sodium nitrite only.

significant increased number of avoidances on the 3rd and 4th day of the learning session compared to the group treated with sodium nitrite only. No significant differences were observed in the memory retention tests (Fig. 1).

In the active avoidance test, the control group showed no significant differences in the number of unconditioned stimuli responses, that is, escapes during the 5 days of the learning session compared to the 1st day. The rats with hypoxia showed significantly decreased number of escapes on the 1st, 2nd, and 3rd day of the learning session compared to the same day controls. The groups subjected to hypoxia and receiving both doses of donepezil had no significantly change in the number of escapes in the learning session. The animals treated with sodium nitrite and 0.5 mg/kg galantamine had significantly increased number of escapes ($P<0.01$) on the 1st day and ($P<0.05$) on the 2nd day of the learning session compared to the same day rats treated with sodium nitrite (Table 1).

In the shuttle box test, the control rats showed significantly increased the number of intertrial crossings ($P<0.05$) on the 5th day compared to the 1st day of the learning session. The group treated with sodium nitrite showed no significant change in the number of intertrial crossings. The animals subjected to hypoxia and receiv-

**Table 2.** Effects of galanthamine and donepezil on active avoidance test (shuttle box) in a model of hypoxia: number of intertrial crossings

| Days | Control Saline 0.1 ml/100 g | $NaNO_2$ 50 mg/kg + Saline 0.1 ml/100 g | $NaNO_2$ 50 mg/kg + donepezil 0.5 mg/kg | $NaNO_2$ 50 mg/kg + donepezil 1.0 mg/kg | $NaNO_2$ 50 mg/kg + galanthamine 0.5 mg/kg | $NaNO_2$ 50 mg/kg + galanthamine 1.0 mg/kg |
|---|---|---|---|---|---|---|
| 1 | 11.00 ± 0.91 | 7.25 ± 2.68 | 8.63 ± 2.30 | 7.13 ± 1.42 | 13.25 ± 1.51* | 5.25 ± 1.66 |
| 2 | 11.00 ± 3.06 | 8.75 ± 3.86 | 11.25 ± 2.69 | 6.50 ± 2.35 | 10.88 ± 4.03 | 6.75 ± 2.41 |
| 3 | 14.63 ± 3.39 | 14.63 ± 4.43 | 31.13 ± 7.48* | 6.75 ± 2.38 | 9.00 ± 2.90 | 4.63 ± 2.53* |
| 4 | 15.88 ± 3.73 | 14.38 ± 5.46 | 20.50 ± 5.40 | 13.25 ± 4.28 | 7.88 ± 2.79 | 4.13 ± 2.05* |
| 5 | 19.63 ± 4.12° | 17.13 ± 5.65 | 16.63 ± 4.62 | 5.38 ± 2.09* | 14.50 ± 3.28 | 9.50 ± 3.32 |
| 12 | 11.50 ± 1.60 | 12.38 ± 4.31 | 7.63 ± 2.44 | 7.13 ± 2.35 | 11.75 ± 2.12 | 5.38 ± 2.27 |

°$P$<0.05, compared to the 1st day control; *$P$<0.05, compared to the same day of the group treated with sodium nitrite only.

**Table 3.** Effects of galanthamine and donepezil on the step-through passive avoidance test in a model of hypoxia: latency of reactions (in seconds)

| Days | Control Saline 0.1 ml/100 g | $NaNO_2$ 50 mg/kg + Saline 0.1 ml/100 g | $NaNO_2$ 50 mg/kg + donepezil 0.5 mg/kg | $NaNO_2$ 50 mg/kg + donepezil 1.0 mg/kg | $NaNO_2$ 50 mg/kg + galanthamine 0.5 mg/kg | $NaNO_2$ 50 mg/kg + galanthamine 1.0 mg/kg |
|---|---|---|---|---|---|---|
| 1 | 72.52 ± 14.84 | 135.96 ± 15.75 | 82.49 ± 15.08 | 68.31 ± 14.47 | 123.75 ± 17.17 | 120.32 ± 15.21 |
| 2 | 129.02 ± 13.86 ° | 147.96 ± 13.92 | 157.81 ± 13.82 | 138.02 ± 15.87 | 153.76 ± 13.26 | 160.38 ± 10.32 |
| 3 | 178 ± 0 ° | 163.27 ± 10.64 | 169.32 ± 8.68 | 155.33 ± 12.34 | 178 ± 0 | 161.31 ± 11.45 |
| 10 | 106.24 ± 16.90 | 159.86 ± 10.25 | 155.57 ± 11.23 | 155.85 ± 12.03 | 142.66 ± 13.16 | 165.43 ± 8.65 |

°$P$<0.05, compared to the 1st day control.

ing 0.5 mg/kg donepezil had significantly increased the number of intertrial crossings ($P$<0.05) on the 3rd day of the learning session compared to the group treated with sodium nitrite only. The hypoxic group treated with 1.0 mg/kg donepezil had a significantly decrease in the number of intertrial crossings ($P$<0.05) on the 5th day of the learning session compared to the sodium nitrite group. The animals treated with sodium nitrite and 0.5 mg/kg galantamine had significantly increased number of intertrial crossings ($P$<0.05) on the 1st day of the learning session. The animals subjected to hypoxia and given 1.0 mg/kg galantamine had significantly decreased number of intertrial crossings ($P$<0.05) on the 1st, 3rd, and 4th day of the learning session compared to the nitrite group rats. No significant differences between the experimental groups in memory retention test were found (Table 2).

In the step-through passive avoidance test, the control rats increased the latency of reaction ($P$<0.05) on the 2nd day of the learning session and in the short memory retention tests (3rd day), compared to the 1st day control. The animals subjected to hypoxia and receiving donepezil or galantamine had no change in the latency of reactions in the learning, short, and long memory retention tests compared to the sodium nitrite group (Table 3).

In the step-down test the control rats had increased latency of reaction in the short memory retention test ($P$<0.05) compared to the 1st day of the learning session. The rats treated with nitrite only had increased latency of reactions ($P$<0.05) in the learning session and preserved it during the long memory retention test compared to the controls. The hypoxic groups injected with both doses of donepezil had significantly increased ($P$<0.05) latency of reactions in the short memory retention test compared to the nitrite group. In the long memory retention test only rats treated with nitrite and donepezil (0.5 mg/kg) showed a significant increase in the latency of reaction ($P$<0.05) compared to the nitrite group. The groups treated with nitrite and both doses galantamine had significantly increased ($P$<0.05) latency of reactions on the 2nd day of the learning session and in the short or long memory retention tests compared to the same day hypoxic rats (Fig. 2).

## Discussion

In the active avoidance test, the control rats showed well-expressed learning ability that was preserved during the memory retention test, suggesting formation of memory traces. The animals in hypoxia showed poor learning and impaired memory paradigm.

In the passive avoidance tests (step-through and step-down), the controls increased the latency of reactions in the learning and short-memory test, that is, they learned the task. The rats of the hypoxia model did not learn

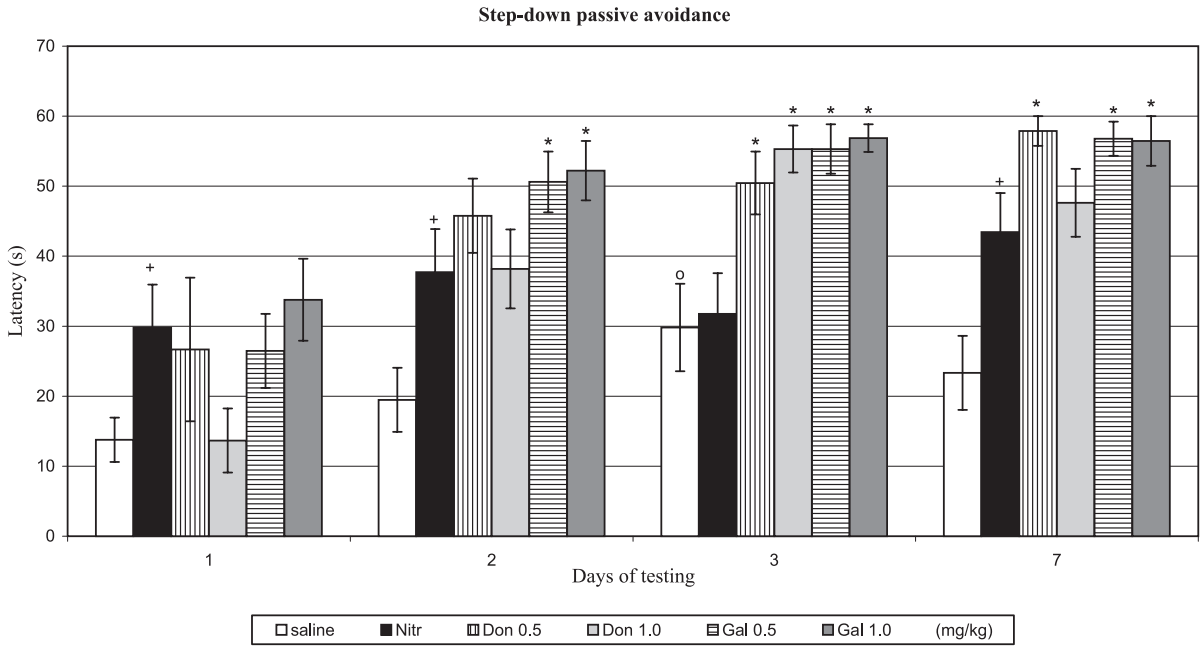


**Fig. 2.** Effects of galantamine (Gal) and donepezil (Don) on latency in step-down passive avoidance test. On the abscissa – days of testing; on the ordinate – latency of time, spent on the platform in seconds. $^{o}P<0.05$, compared to the 1st day control; $^{+}P<0.05$, compared to the same day control; $*P<0.05$, compared to the same day rats treated with sodium nitrite only.

the task (step-through) and had increased latency of reactions (step-down) in the learning test and in the long memory retention, probably due to decreased motor activity induced by sodium nitrite. The chronic application of sodium nitrite induced long-term consequences, mainly impaired behavior (9), suggesting that this increased latency is probably not a real memory improvement.

The histochemical study showed that the development of cholinergic innervation after long-term prenatal hypoxia (induced by chronic exposure to sodium nitrite) was impaired. The animals had a transient delay in the cholinergic innervation of parietal neocortex and dental gyrus and retardation of the cholinergic fiber development and outgrowth in cortical target area (11). The reduced learning ability and impaired memory in our experiments were probably due to induced by sodium nitrite hypoxia leading to a decreased oxygen content in the brain as suggested by Kožiar et al. (12).

There are data that hemic hypoxia caused by the methemoglobin-inducing agent sodium nitrite evokes disturbed habituation in the open field and impaired learning and memory in the passive avoidance paradigm (12). Other authors (13) have studied the short-term effects and long-term consequences of sodium nitrite hypoxia on the spontaneous behavior of rats. They found delayed behavioral deficits, which could be due to the secondary structural alterations in the CNS due to exposure to sodium nitrite for several days. Our results are in accord with such suggestions.

In our experiments, the anticholinesterase drugs donepezil and galantamine that have stimulating effect on CNS improved the active avoidance learning in the model of hypoxia. Donepezil antagonized the damaging effect of sodium nitrite and improved learning ability in the last two days of the learning period. Galantamine also abolished the effect of sodium nitrite in the rats. The rats that were given donepezil or galantamine showed stimulated motor activity (increased the number of intertrial crossings) in the learning, but not in the memory retention test.

Neither donepezil nor galantamine improved learning and memory retention in the passive avoidance tests in rats subjected to hypoxia. Donepezil did not change learning and memory retention in the hypoxic rats. Galantamine improved learning and short and long-term memory retention in the step-down passive avoidance test.

Donepezil and galantamine are different in their chemical structure. Both drugs are metabolized by the microsomal cytochrome $P_{450}$ system in the liver. There are data that donepezil inhibits acetylcholinesterase, while galantamine not only inhibits acetylcholinesterase, but has also an allosteric modulation of the N-cholinergic receptors (5). This allosteric modulation of the N-cholinergic receptors means that the same amount

of acetylcholine is able to produce more current postsynaptically in a concentration-dependent inverted U curve (14, 15). Our data allow such speculation because of the differences in the effects of both cholinesterase inhibitors galantamine and donepezil and the fact that the second compound did not act as a ligand of this receptor subtype.

Some data suggest that chronic low-level stimulation of nicotinic receptors may up-regulate their expression and slow down neurodegeneration (16). Galantamine by way of allosterically potentiating ligand action could provide a sustained level of chronic stimulation, thereby helping to restore healthy nicotinic receptor expression levels. Similarly, there is initial evidence that chronic low-level stimulation of N-cholinergic receptors protects against $\beta$-amyloid toxicity by increased release of the terminally truncated secreted form of $\beta$-amyloid protein production (17).

Haŭg et al. (18) found that after 14 days administration of 3 mg/kg donepezil the cerebral acetylcholine level was increased by 35% and the cholinesterase activity was decreased by 66% in brain. Others (19) pointed out that donepezil improves the function of learning and memory of rats and the mechanism is associated with its recovering the hippocampal structure and function of synapse and enhancing its plasticity.

Our results taken together with literature findings allow us to conclude that in the rat model of hypoxia, both anticholinesterase drugs have improving effects. The difference is that donepezil has better expressed effect in the active avoidance test and galantamine has a better effect in the passive avoidance test, probably because of their subtle differences in their mechanisms of action.

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