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Comparative X-Ray study of galantamine and tacrine on the evacuatory function of rat gastrointestinal tract

Research article

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Abstract: A The acetylcholinesterase inhibitors galantamine and tacrine are used to treat Alzheimer's disease. However, these compounds also affect the gastrointestinal (GI) tract. Here, we compared and analyzed both the effects of galantamine- and tacrine on the evacuatory kinetics of the GI tract in rats. Rats were untreated (n=15) or treated with galantamine (one daily dose of 1 mg/kg per os for 21 days; n=17) or tacrine (one daily dose of 0.5 mg/kg per os for 21 days; n=13) and evacuatory kinetics were assessed using radiological methods. Galantamine initially slowed and then accelerated evacuation, which is characteristic of the majority of cholinesterase inhibitors and is a result of the endogenous acetylcholine accumulated in the GI tissues. In the tacrine-treated rats the contrast medium was kept in the stomach and cecum and its evacuation time was reliably increased. These results indicate that when administered for 20 days, galantamine and tacrine have different effects on motor and evacuatory function in the GI tract of rats, because at certain levels of the tract the tacrine-action is dominated by specific non-cholinergic and non-anticholinesterase mechanisms.

Keywords: *Gastrointestinal tract • Evacuatory kinetics • Galantamine • Tacrine*

1. Introduction

Galantamine and tacrine are acetylcholinesterase inhibitors used for the symptomatic treatment of Alzheimer's disease and other forms of dementia due to cholinergic deficit [1-5]. Galantamine is a tertiary alkaloid that is a reversible, competitive inhibitor of acetylcholinesterase [6]. It also interacts allosterically with nicotinic (N-) acetylcholine receptors, enhancing the action of receptor agonists [7]. Furthermore, galantamine is a selective inhibitor of acetylcholinesterase and is a weak inhibitor of butyrilcholinesterase [8]. Tacrine is a derivate of acridine and is a reversible and non-competitive inhibitor of acetylcholinesterase with a complex mechanism of action [1,2,9,10]. Tacrine is also a stronger inhibitor of butyrilcholinesterase than galantamine [1] and, at doses used to improve central nervous system function, it has a stronger effect on peripheral structures, such as the gastrointestinal (GI) tract. Some of the effects of tacrine are due to the inhibition of certain subtypes of cholinergic receptors [11,12]. Tacrine has been reported to induce tissue-dependent down-regulation of muscarinic (M-) cholinergic receptors [13]. Tacrine also blocks transmembrane K^+ flow [14] and influences Ca^{2+} homeostasis [15]. The non-cholinergic and non-anticholinesterase effects of tacrine are known to occur at doses higher than those used therapeutically [16].

Galantamine and tacrine have some side-effects due their action on organs and systems outside the

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central nervous system. The most common are in the GI tract, namely, abdominal pain [17], nausea, vomiting [18,19,20] and diarrhea [17,21]. Also, long-term therapy with tacrine has been reported to cause a loss of appetite, resulting in weight loss [16]. According to Davis and Powchik, between 2% and 29% of patients treated with tacrine and between 7% and 25% of those treated with galantamine develop GI side-effects [16]. Most of these side-effects are due to disruption of gastric or intestinal motility.

In the current study, we analyzed the characteristics of galantamine and tacrine induced on the evacuatory function of the rat GI tract.

2. Material and Methods

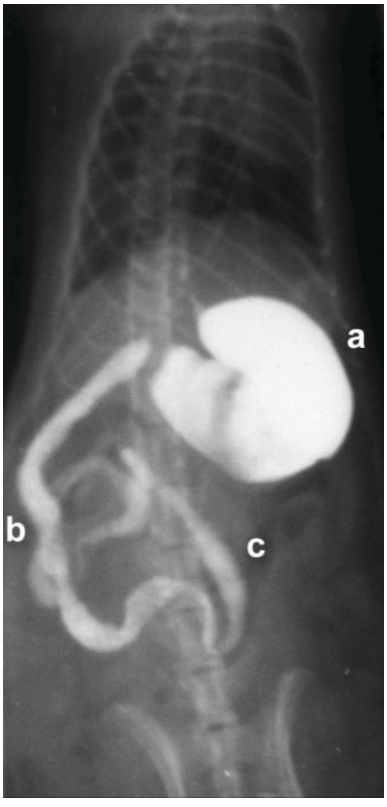
2.1. Experimental animals

The experiments were carried out using 45 adult male Wistar rats (weight, 290±20 g). Throughout the experiment (21 days), the animals were kept under standard laboratory conditions, including a food pellet diet, temperature, dark/light-regime, and free access to water. The animals were divided into three groups: group 1 (n=15), controls, which were given saline; group 2, galantamine-treated (n=17), which received a single daily dose of 1 mg/kg galantamine per os for 21 days; and group 3, tacrine-treated (n=13), which were treated with a single daily dose of 0.5 mg/kg tacrine per os for days. These doses are galantamine and tacrine are used therapeutically, and we have shown that they are equally effective in behavior experiments. Sixteen hours prior to performing radiographic examination of the animals' GI tracts, they were denied access to food and were about 70% deprived to water.

2.2. X-ray contrast examination

Contrast facial radioscopy, immediately followed by radiographs of the GI tract, were performed using a Sirescop 1500 ST X-ray apparatus (Siemens) with a constant focus distance of 0.7 m. Animals were administered 2.5 ml of contrast medium (CM; 120 mg $BaSO_4$ dissolved in 200 ml H_2O at 37°C) per os. Radiographs were made immediately and 1, 3, 6, 12, 24, and 28 h after administration of the CM. The time for the complete evacuation of the CM out of the stomach and the intestinal passage after 24 h were used as quantitative indices. The differences in the shape, size, tone, and peristaltic activity of the stomach and intestine in all animal groups were also assessed. All experiments were performed in accordance with requirements for work with laboratory animals were observed, as stated in the European convention. The experiments were also approved by Ethical Committee of the Medical University Plovdiv (Protocol No. 37/12.04.2005).

Figure 1. Contrast facial radiographs from a control rat immediately after an administration of CM. CM is present in the stomach (a), duodenum (b), and part of jejunum (c).



2.3. Drugs and substances

Galantamine was from Tocris, tacrine was from Sigma, and $BaSO_4$ was from Sopharma.

2.4. Statistical analysis

Continuous variables are expressed as means ± SEM. The statistical association between the time of evacuation of CM from the stomach and the GI tract for controls, galantamine-treated, and tacrine-treated rats was assessed by analysis of variance using Tukey's HSD post-hoc test. Because CM was found in the cecum and also in the stomach of some tacrine-treated rats. For this reason at the final (28 h) time point, 28 h was accepted as a KM time-an indicator of CM evacuation value for these animals. Because of the small sample size, the experimental results from analysis of variance were also assessed using the nonparametric Kruakal-Wallis test. A value of $P < 0.05$ was considered to indicate a significant difference.

Figure 2. A control rat 24 h after CM administration. Some of the CM is present in the large bowel.

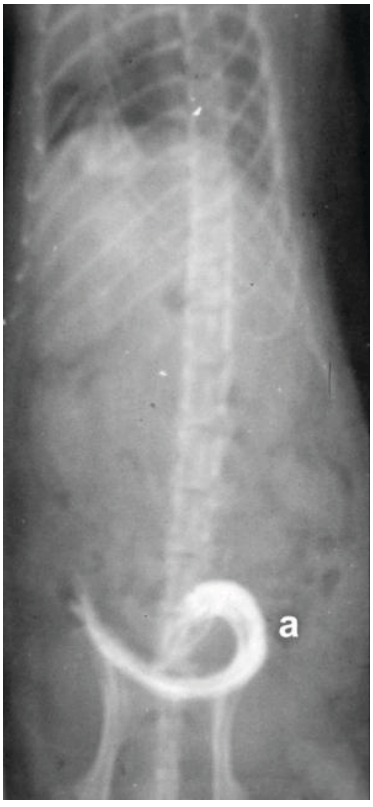
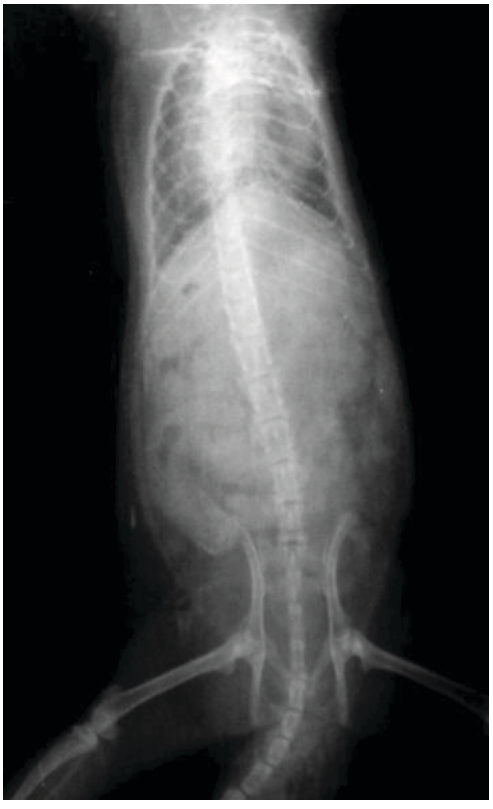


Figure 3. A galantamine-treated rat immediately after CM administration. Typical alternate segments with narrowed (a) and widened (b) lumen in the small intestines are observed.



3. Results

3.1. Characteristics of the GI tract and its motor and evacuatory function in control rats

We first examined the stomachs and intestines of control rats. We found that they were of normal sizes and shapes, their anatomic parts were clearly discernible, and peristaltic waves were deep and symmetric. Immediately after the introduction of CM, it filled the stomach, duodenum, and the initial part of the jejunum (Figure 1). One hour later, it was evacuated from the stomachs of 11 of the 15 control rats and was distributed in the small bowels. After 3 h, the CM was evacuated from the stomachs of all but one of the control rats. After 6 h, the CM was evacuated from the small bowels and started filling the cecum in all but four animals. After 12 h, the CM was distributed throughout the large bowel. Finally, after 24 h, the CM was completely evacuated from six of the animals, and in the remaining nine, traces of the CM could be observed in the rectum. These observations are shown in Figure 2.

3.2. Effects of galantamine on the condition, motility and evacuatory activity of the GI tract

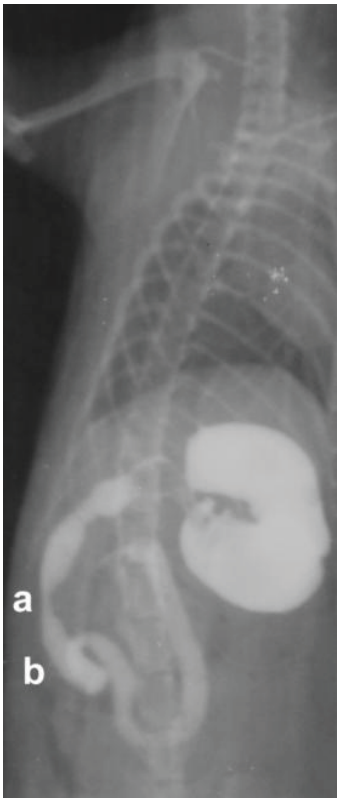
We next examined the GI tract of the galantamne-treated rats. Their stomachs were of regular shape, location, with clearly demarcated outlines and well-differentiated antral and fornical parts. The radiographic images demonstrated some variation in the CM distribution and movement. Typically, there were alternate segments of narrowed and widened lumen throughout of the duodenum, and in some animals, this was also visible in the jejunum (Figure 3). X-radiographs revealed peristaltic activity in the ileal area. After 6 h, the CM was evacuated from the stomach of all glantamine-treated rats. Radiographs taken after 12 h showed CM in the rectum, and after 18 h, it was completely evacuated from eight animals. Ater 24 h, traces of CM could not be found in the GI tract in any of the rats (Figure 4). Of the 17 galantamine-treated rats, 13 had diarrhea on the day of CM administration.

Table 1. Kinetics of CM evacuation in the rat gastrointestinal tract.

Index	Control group (n=15)	Galantamine-treated group (n=17)	Tacrine-treated group (n=13)
Complete stomach evacuation, h	1.53 ± 0.24	4.47 ± 0.42	26.77 ± 0.53
		$P < 0.001$*	$P < 0.001$*
			$P < 0.001$†
Complete GI tract evacuation, h	25.07 ± 1.89	19.76 ± 1.00	28.00 ± 0.00
		$P < 0.001$*	$P = 0.054$*
			$P < 0.001$†

** Comparison between experimental group and control*
† Comparison between galantamine- and tacrine-treated groups

Figure 4. A galantamine-treated rat 24 h after CM administration. Traces of CM could not be found in the GI tract.



3.3. Effects of tacrine on the condition, motility and evacuatory activity of the GI tract

Finally, we examined the effects of tacrine on the GI tract in rats. Of the 13 tacrine-treated rats, 9 had enlarged stomachs. The widening involving mainly the fornix. The stomachs were contracted in their antral area. CM could be observed only in the stomach. Radiographic images did not reveal peristaltic waves (Figure 5). The stomachs of the remaining rats were normal in size, and they had some peristaltic activity. Three hours after administration, CM was localized mainly in the fornix of the stomach in seven rats, and in the remaining six, it was evenly distributed throughout stomach, duodenum, and small bowels. The location of the bowels of most animals was preserved, and they had a normal lumen. After 6 h, the fornix and cecum of eight rats were filled with CM. The cecum was hypotonic. After 12 h, all of the had CM in the fornical part of the stomach, and 9 of them in the cecum as well. After 24 h, partial evacuation of CM from the digestive tract was observed in eight animals. The feces was of normal form and consistency. The CM that remained in the intestinal tract was limited to the stomach and cecum (Figure 6).

The quantitative radiological differences of the GI function in the different groups was assessing by ANOVA (Table 1). The Kruskal-Wallis test was used to confirm the significance of differences among the groups.

4. Discussion

We show heere that a 21-day treatment with galantamine or tacrine causes functional disturbances in the GI tract of rats. Acetylcholine stimulates tonus and motility of the GI tract [22]. The mechanism of these drugs in the GI must be similar because they had similar effects on the kinetics of evacuation.

The muscle tone of the GI tract in the galantamine-treated rats was increased compared to controls. This is likely mediated by acetylcholine because contraction of smooth muscle from various areas of the GI tract by galantamine is blocked by the non-specific M-cholinergic antagonists atropine and ipratropium [23]. The effects of galantamine and of the accumulated acetylcholine, however, are not identical in the different areas of the GI tract. This may be due to the heterogeneous distribution of different cholinergic receptor subtypes along the GI tract, or it may be related to certain specific contractile characteristics of the muscles in the various areas.

Figure 5. Contrast facial radiographs of a tacrine-treated rat immediately after administration of CM. CM is present only in the stomach. (a) shows an enlarged view of the fornical part. The antral area (b) is contracted. Some peristaltic waves are missing.

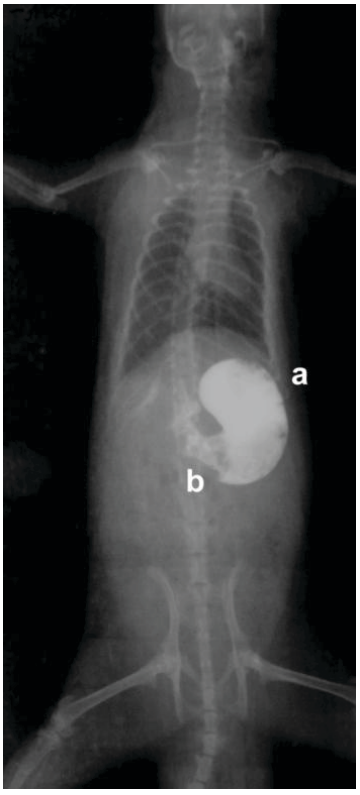
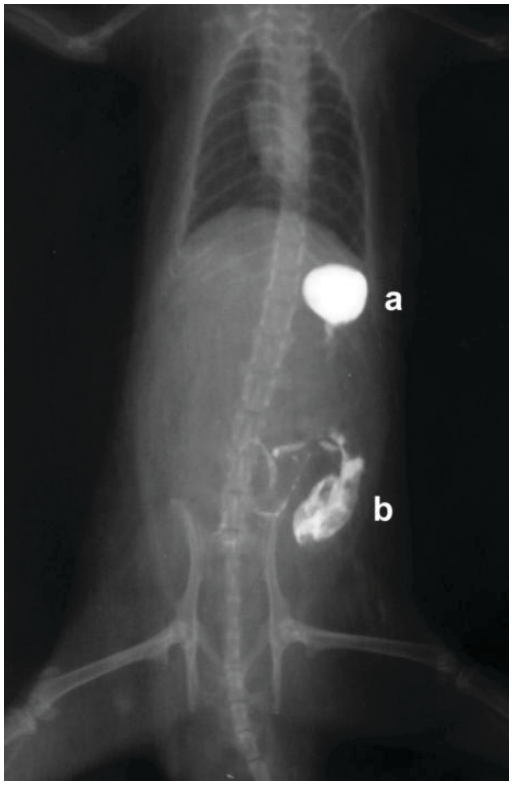


Figure 6. Contrast facial radiographs of a tacrine-treated rat 24 h after administration of CM. $BaSO_4$ is found only in the CM store in the stomach (a) and the cecum (b).



It is also possible that galantamine causes changes in the expression of cholinergic receptors [24] or the sensitivity of N-cholinergic receptors [25,26] or that accumulated acetylcholine affects various types of M- and/or N-cholinergic receptors expressed on enteric inhibitory and excitatory neurons [27] controling GI motility [28]. These effects acting at a deodenal or jejunal region probably cause the appearance of stenotic segments. Such regions of decreased lumen possess increased tonus and resist the passage of CM to the lower levels of the GI tract. This increased resistance in the duodenum is responsible for the lack of contrast in the small bowels in most of the galantamine-treated rats and for the slowing of the passage at that level of tract.

In vitro experiments show that galantamine selectively stimulates the spontaneous phasic activity of smooth muscle tissues from rat's ileum [23]. The increased phasic activity is apparently required for the increase in peristaltic activity in the ileum that was observed in most of the animals. Furthermore, this increased activity, combined with the overall increase in the tonus of the tract, is probably what speeds the intestinal passage after the sixth hour in galantamine-treated rats. This results in the complete evacuation of the CM in half of the galantamine-treated animals as early as the 18th hour as well as the significant reduction in the evacuation time compared to controls. The loose consistency of the feces in the galantamine-treated animals can be explained by the accelerated passage.

Like galantamine, tacrine is a reversible inhibitor of acetylcholinesterase [29]. However, the tacrine-treated animals showed some specific reactions, namely, fornical and caecal relaxation, antral contraction, and a lack of statistically significant peristaltic activity. The combination of tacrin-induced non-anticholinesterase and non-cholinergic tonic relaxation and the inhibition of the phasic mechanical activity [30] combined with the contractile effects of acummulated actylcholine can account for the changes that we observed. In addition, there are likely variations in the thresholds for these acetylcholinesterase-indepent effects in different areas of the GI tract [31]. In some areas

of the GI tract, tacrine-induced reactions are due mostly to non-cholinergic mechanisms, whereas in others, cholinergic mechanisms dominate.

The widening of the fornical area most likely indicates that there is decreased intragastric pressure. This combined with the observed spasm of the antral muscles results in incomplete evacuation of the CM from the stomachs and formation of a gastric depot. This combination of factors also causes the formation of a depot in the cecum. The partial inhibition of peristaltic activity in tacrine-treated rats, the hypotonia and formation of depots in parts of the stomach and cecum, the slowing of CM evacuation, and the lack of diarrhea do not appear to be directly related to the accumulation of acetylcholine.Thus, it appears that both anticholinesterase-dependent and -independent mechanisms are involved in the GI side-effects of tacrine. These effects are observed a dose similar to those used therapeutically. In this way, the effect of tacrine on the motor and evacuatory function of the GI tract is distinct from those of galantamine and some other anticholinesterase inhibitors.

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References

[1] Rakonczay Z., Potencies and selectivity of inhibitors of acetylcholinesterase and its molecular forms in normal and Alzheimer's disease brain, Acta Biol. Hung., 2003, 54, 183-189

[2] Wong W.J., Liu C.H., Fuh J.L., Wang S.J., Hsu L.C., Wang P.N., et al., Double-blind, placebo-controlled study of tacrine in Chinese patients with Alzheimer's disease, Dement. Geriatr. Cogn. Disord., 1999, 10, 289-294

[3] Scott L.J., Goa K.L., Galantamine: a review of its use in Alzheimer's disease, Drugs, 2000, 60, 1095-1122

[4] Corey-Bloom J., Galantamine: a review of its use in Alzheimer's disease and vascular dementia, Int. J. Clin. Pract., 2003, 57, 219-223

[5] Raskind M.A., Sadowsky C.H., Sigmund W.R., Beitler P.J., Auster S.B., Effect of tacrine on language, praxis, and noncognitive behavioral problems in Alzheimer disease, Arch. Neurol., 1997, 54, 836-840

[6] Zarotsky V., Sramek J.J., Cutler N.R., Galantamine hydrobromide: an agent for Alzheimer's disease, Am. J. Health. Syst. Pharm., 2003, 60, 446-452

[7] Maelicke A., Allosteric modulation of nicotinic receptors as a treatment strategy for Alzheimer's disease, Dement. Geriatr. Cogn. Disord., 2000, 11, 11-18

[8] Darvesh S., Walsh R., Kumar R., Caines A., Roberts S., Magee D., et al., Inhibition of Human Cholinesterases by Drugs Used to Treat Alzheimer Disease, Alzheimer Dis. Assoc. Disord., 2003, 17, 117-126

[9] Stemmelin J., Cassel J., Will J.C., Kelche B.C., Sensitivity to cholinergic drug treatments of aged rats with variable degrees of spatial memory impairment, Behav. Brain Res., 1999, 98, 53-66

[10] Ventura M., Sternon J., Therapeutic results: tacrine, Rev. Med. Brux., 1997, 18, 394-397

[11] Hunter A., Murray T., Jones J., Cross A., Green A., The cholinergic pharmacology of tetrahydroaminoacridine in vivo and in vitro, Br. J. Pharmacol., 1989, 98, 79-86

[12] Snape F., Misra A., Murray T., De Souza R., Williams J., Cross A., et al., A comparative study in rats of the in vitro and in vivo pharmacology of the acetylcholinesterase inhibitors tacrine, donepezil and NXX-066, Neuropharmacol., 1999, 38, 181-193

[13] Flynn D., Mash D., Multiple in vitro interactions with and differential in vivo regulation of muscarinic receptor subtypes by tetrahydroaminoacridine, J. Pharmacol. Exp. Ther., 1989, 250, 573-581

[14] Summers W., Majovski L., Marsh G., Tachiki K., Kling A., Oral tetrahydro-aminoacridine in long-term treatment of senile dementia, Alzheimer type, N. Engl. J. Med., 1986, 315, 1241-1245

[15] Dolezal V., Lisa V., Tucek S., Effect of tacrine on intracellular calcium in cholinergic SN56 neuronal cells, Brain Res., 1997, 769, 219-224

[16] Davis U.L., Powchik P., Tacrine, Lancet, 1995, 345, 625-630

[17] Nordberg A., Svensson A.L., Cholinesterase inhibitors in the treatment of Alzheimer's disease: a comparison of tolerability and pharmacology, Drug Safety., 1999, 20, 146

[18] Sramek J.J., Frackiewicz E.J., Cutler N.R., Review of acetylcholinesterase inhibitor galantamine, Exp. Opin. Invest. Drugs, 2000, 9, 2393-2402

[19] Poirier J., Evidence that the clinical effects of cholinesterase inhibitors are related to potency and targeting of action, Int. J. Clin. Pract. 2002, 127, 6-19

[20] Fulton B., Benfield P., Galanthamine, Drugs Aging., 1996, 9, 60-67

[21] Cummings J.L., Use of cholinesterase inhibitors in clinical practice: evidence-based recommendations, Am. J. Geriatr. Psych., 2003, 11, 131-145

[22] Gerthoffer W.T., Signal-transduction pathways that regulate visceral smooth muscle function. III. Coupling of muscarinic receptors to signaling kinases and effector proteins in gastrointestinal smooth muscles, Am. J. Physiol. Gastrointest. Liver Physiol., 2005, 288, G849-G853

[23] Turiiski V.I., Krustev A.D., Sirakov V.N., Getova D.P., In vivo and in vitro study of the influence of the anticholinesterase drug galantamine on motor and evacuative functions of rat gastrointestinal tract, Eur. J. Pharmacol., 2004, 498, 233-239

[24] Barnes C.A., Meltzer J., Houston F., Orr G., McGann K., Wenk G.L., Chronic treatment of old rats with donepezil or galantamine: effects on memory, hippocampal plasticity and nicotinic receptors, Neuroscience, 2000, 99, 17-23

[25] Maelicke A., Samochocki M., Jostock R., Fehrenbacher A., Ludwig J., Albuquerque E.X., et al., Allosteric sensitization of nicotinic receptors by galantamine, a new treatment strategy for Alzheimer's disease, Biol. Psych, 2001, 49, 279-288

[26] Coyle J., Kershaw P., Galantamine, a cholinesterase inhibitor that allosterically modulates nicotinic receptors: effects on the course of Alzheimer's disease, Biol. Psych., 2001, 49, 289-299

[27] Vizi E.S., Kobayashi O., Torocsik A., Kinjo M., Nagashima H., Manabe N., et al., Heterogeneity of presynaptic muscarinic receptors involved in modulation of transmitter release, Neuroscience, 1989, 31, 1259-267

[28] Brookes S.J., Neuronal nitric oxide in the gut, J. Gastroenterol. Hepatol., 1993, 8, 590-603

[29] Jeyarasasingam G., Yeluashvili M., Quik M., Tacrine, a reversible acetylcholinesterase inhibitor, induces myopathy, Neuroreport, 2000, 11, 1173-1176

[30] Kristev A.D., Argirova M.D., Getova D.P., Turiiski V.I., Prissadova N.A., Calcium-independent tacrine-induced relaxation of rat gastric corpus smooth muscles, Can. J. Physiol. Pharmacol., 2006, 84, 1133-1138

[31] Turiiski V., Argirova M., Krastev A., Non-anticholinesterase, non-cholinergic effect of tacrine on gastrointestinal smooth muscle tissues of rat, Folia Medica, 2005, 57, 45-51