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*Short Communication***Synergistic Effects of Galantamine and Memantine in Attenuating Scopolamine-Induced Amnesia in Mice**Perrine Busquet¹, Valeria Capurro¹, Andrea Cavalli^{1,2}, Daniele Piomelli^{1,3}, Angelo Reggiani¹, and Rosalia Bertorelli^{1,*}¹*Drug Discovery and Development, Fondazione Istituto Italiano di Tecnologia, Via Morego 30, 16163 Genoa, Italy*²*Department of Pharmaceutical Sciences, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy*³*Departments of Pharmacology and Biological Chemistry, University of California, Irvine, CA 92697-4621, USA*

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Abstract. We investigated a possible drug efficacy enhancement obtained by combining inactive doses of galantamine and memantine in the scopolamine-induced amnesia model in mice. We evaluated the effects of the two drugs, either alone or in combination, using the spontaneous alternation and object recognition tasks. In both tests, combination of low doses of galantamine (0.1 mg/kg, s.c.) and memantine (0.5 mg/kg, i.p.), which were sub-active *per se*, rescued the memory impairment induced by scopolamine (1 mg/kg, i.p.). The results suggest that combinations of galantamine and memantine might provide a more effective treatment of memory impairments in cognitive disorders than either drug used alone.

Keywords: neurodegenerative disease, multi-target, memory impairment

Alzheimer's disease (AD) is a progressive neurological disorder characterized by deterioration of cognitive function, dementia, memory loss, and altered behavior. Disrupted cholinergic and glutamatergic mechanisms have been hypothesized to be crucial for the development of the disease and preclinical and pathologic evidence support this hypothesis. The first drugs approved by the United States Food and Drug Administration to treat memory impairment in mild to moderate AD were acetylcholinesterase inhibitors (AChEIs) such as galantamine, donepezil, and rivastigmine. Drugs targeting *N*-methyl-D-aspartate (NMDA) receptors are an additional focus in the pharmacological therapy of AD, and memantine, an uncompetitive NMDA antagonist, has also been approved for use as monotherapy in patients with moderate to severe AD. Unfortunately, these drugs provide statistically significant but clinically modest benefits over placebo with respect to cognition, daily function, and behavior in patients, in particular over long-term treatment periods of time (1). In addition, the side effects caused by these agents, due to increasing dosages as the disease progresses, are notable and include gastrointesti-

nal problems, anxiety, and insomnia (2, 3). Therefore, a rationale for combination therapy of galantamine and memantine has been proposed. Clinical studies have supported this proposal, indicating that such a combination may provide better outcomes and produce fewer side effects when compared to monotherapy (1).

In the present report, we investigated a possible drug efficacy enhancement obtained by combining inactive doses of galantamine and memantine. Experiments were carried out in mice, using the scopolamine-induced amnesia model and evaluating drug efficacy with the spontaneous alternation and the object recognition tests. We found that combinations of inactive doses of galantamine and memantine resulted in a significant cognitive enhancement. The results support efforts aimed at discovering new dual-acting molecules that integrate the pharmacological activities of AChEIs and memantine.

For all studies, CD1 male mice (18–22 g; Charles River Laboratories, Calco, Italy) were group-housed in a temperature and humidity controlled room under a 12-h light/dark cycle (light on at 7 a.m.) with water and food *ad libitum*. At least 24 h before testing, animals were brought to the experimental room and kept in a ventilated storage cabinet (Tecniplast, Buguggiate, Italy). Experimental procedures were performed during the light phase, therefore during the inactive circadian phase of the ani-

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mals. Mice were only tested once. All experiments were conducted in compliance with Italian regulations on protection of animals used for experimental and other scientific purposes (D.M. 116192) as well as with European Economic Community regulations (O.J. of E.C. L 358/1 12/18/1986). Galantamine hydrobromide, scopolamine hydrochloride (Sigma Aldrich, Milano, Italy), and memantine hydrochloride (Tocris Bioscience, Bristol, UK) were dissolved in sterile 0.9% saline. Galantamine and memantine were administered by subcutaneous (s.c.) or intraperitoneal (i.p.) administration, respectively, 30 min before tests. Scopolamine (1 mg/kg) was administered i.p. 20 min before tests. Statistical analyses were performed using One-way ANOVA followed by a post-hoc Tukey's test, using Graph Pad Prism version 5.00 (Graph Pad Software, San Diego, USA). *P*-levels < 0.05 were considered statistically significant.

Spatial memory performance was assessed using the T-maze task, which was carried out according to Spowart-Manning and van der Staay (4) with slight modifications. The maze (Ugo Basile, Comerio, Italy) was made of a gray, non-reflective base plate and plastic arms (28 × 5 × 10 cm). Illumination on the floor of the table was approximately 150 lux. Training consisted of one session, which started with one forced-choice trial, followed by 14 free-choice trials. In the first trial, the 'forced-choice trial', either the left or right goal arm was blocked by a cardboard door. The animal was released from the start arm and was allowed to explore the maze, entering the open goal arm, and return to the start position where it was confined for 5 s by lowering the guillotine door. During the following 14 'free-choice' trials and after opening the door, the animal was free to choose between the left and right goal arm. As soon as it entered one goal arm, the other goal arm was closed and once it returned to the start arm, the next free-choice trial started after 5-s restraint in the start arm. A session was terminated and the animal was removed from the maze as soon as 14 free-choice trials were performed or 15 min elapsed. The series of arm entries was recorded visually by investigators blinded to treatment. The percentage of alternations was calculated as (actual alternations / total possible alternations) × 100. The T-maze was cleaned with a 40% ethanol solution after each session. Animals that did not finish the test within 15 min were discarded as considered poorly explorative.

As illustrated in Fig. 1, administration of scopolamine (1 mg/kg, i.p.) caused a significant reduction of the percentage of alternations in the T-maze, compared to vehicle controls. The administration of galantamine, which *per se* did not exert any effect, produced a statistically detectable reduction of the scopolamine-induced deficit at the doses of 0.3 and 1 mg/kg [One-way ANOVA

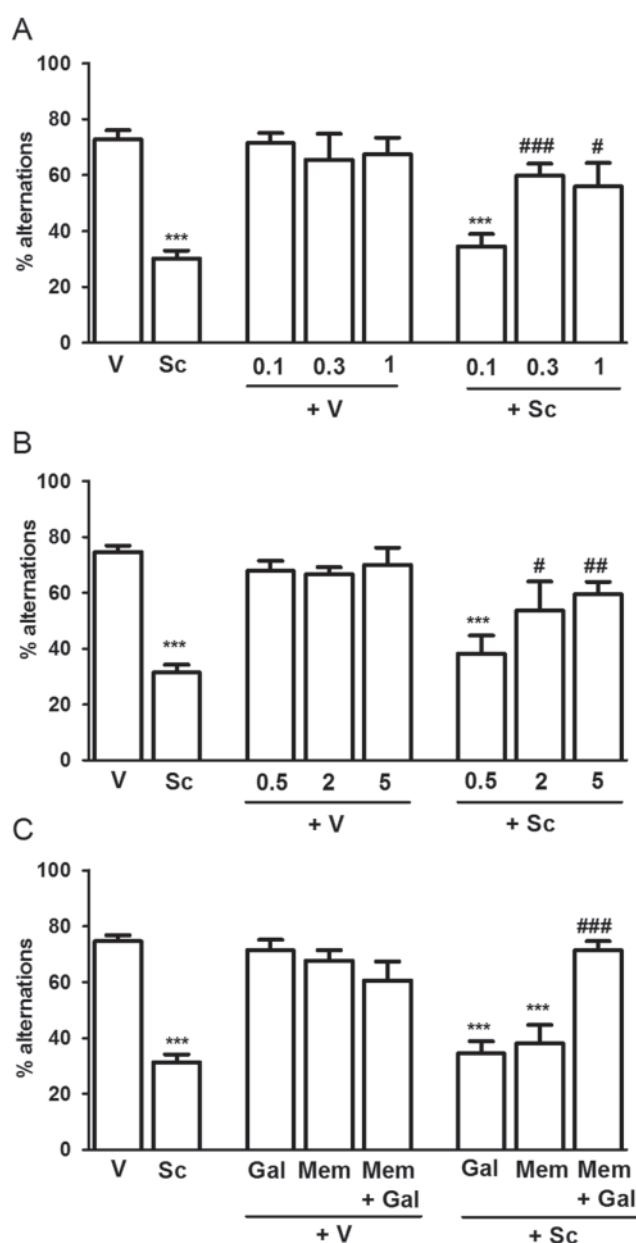


Fig. 1. Effect of galantamine (Gal) (A), memantine (Mem) (B), and their co-administration (C) on spontaneous alternation behavior in the scopolamine-induced amnesia model in mice ($n = 8 - 10$). Galantamine (0.3 – 1 mg/kg, s.c.) and memantine (0.5 – 5 mg/kg, i.p.) were administered 10 min before scopolamine (1 mg/kg, i.p.). Data are expressed as the mean \pm S.E.M. *** $P < 0.001$ vs. controls (V); # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ vs. scopolamine-treated animals (Sc).

$F(7,70) = 14.16, P < 0.0001$] (Fig. 1A). As shown in Fig. 1B, memantine exerted similar memory-enhancing effects, against scopolamine, at 2 and 5 mg/kg [One-way ANOVA $F(7,62) = 15.53, P < 0.0001$]. Importantly, the combination of two inactive doses of galantamine and memantine (0.1 and 0.5 mg/kg respectively), given at the

same time, was able to significantly revert the detrimental effects of scopolamine in this test [One-way ANOVA $F(7,66) = 26.93, P < 0.0001$] (Fig. 1C).

Episodic memory was assessed using the novel object recognition task according to Bevins and Besheer (5), in an open field (OF, plastic box 46.5×43.5 cm; Ugo Basile), under red light and on 2 successive days. On the first day, mice were allowed to explore an empty OF for adaptation. The second experimental day was divided into 2 trials of 5 min each. At 30 and 20 min before the first trial, animals were treated with galantamine and/or memantine and scopolamine. On the first trial (T1), mice were presented with two similar objects ('familiar objects', F). After 15 min, trial 2 (T2) started and mice were exposed to two different objects: F and a novel object (N). Object exploration, defined as the time in which a mouse touched the object with its nose or was oriented toward and within 2 cm to the object, was measured manually by investigators kept unaware of the treatment. Subsequently, the relative measure of object discrimination during T2 was calculated as discrimination index = $(N - F) / (N + F)$. Between each test, the box floor and the objects were cleaned with a 40% ethanol solution. Mice that explored both objects for less than 10 s were discarded.

During adaptation to the OF on day 1, all the animals demonstrated similar motor activity and anxiety levels. Figure 2A shows that galantamine significantly reverted scopolamine-induced amnesia at the 0.3 and 1 mg/kg doses [One-way ANOVA $F(7,90) = 6.530, P < 0.0001$]. Conversely, memantine did not significantly antagonize the scopolamine-induced deficit and a slight non-significant change in memory performance was observed only at the 2 mg/kg dose [One-way ANOVA $F(7,68) = 3.762, P = 0.0019$] (Fig. 2B). However, as illustrated in Fig. 2C, the co-administration of an inactive dose of galantamine (0.1 mg/kg) and the lowest inactive dose of memantine (0.5 mg/kg) produced a statistically detectable memory-enhancing effect [One-way ANOVA $F(7,67) = 8.461, P < 0.0001$].

In the present study, we confirmed that either galantamine or memantine improves, when administered alone, cognitive performance in the model of scopolamine-induced amnesia (6, 7). Our most important finding was, however, that the combination of doses of galantamine (0.1 mg/kg, s.c.) and memantine (0.5 mg/kg, i.p.), which were *per se* inactive, effectively rescued the memory impairments elicited by scopolamine. It is thought that the association of cholinergic and glutamatergic dysfunction, such as nicotinic and NMDA-receptors down-regulation (8, 9), underlies the symptomatology of AD, but only few preclinical studies have examined whether co-administration of these drugs could

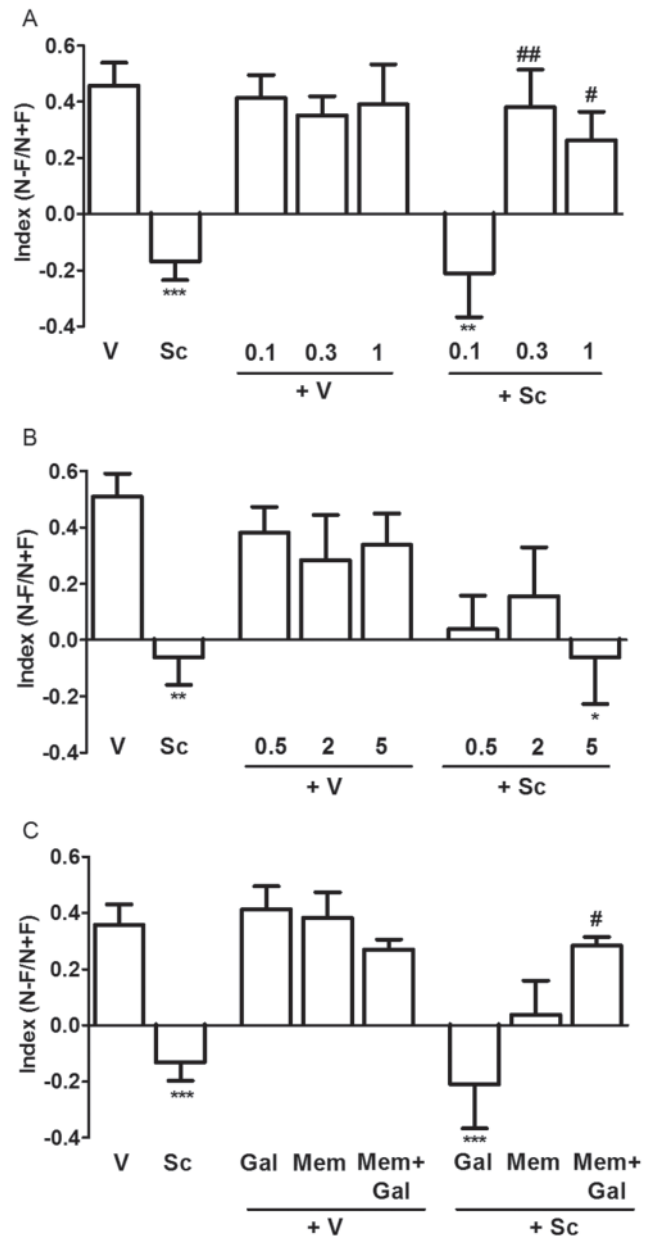


Fig. 2. Effect of galantamine (Gal) (A), memantine (Mem) (B), and their co-administration (C) in the object recognition task using a scopolamine-induced amnesia model in mice ($n = 7 - 10$). Galantamine (0.1 - 1 mg/kg, s.c.) and memantine (0.5 - 5 mg/kg, i.p.) were administered 10 min before scopolamine (1 mg/kg, i.p.). Data are expressed as the mean \pm S.E.M. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. controls (V); # $P < 0.05$, ## $P < 0.01$ vs. scopolamine-treated animals (Sc).

actually enhance memory (10). Our findings indicate that co-administration of sub-threshold doses of memantine and galantamine increases memory performance in scopolamine-impaired mice. The mechanism underlying the synergic interaction between the two drugs is unclear. There are multiple potential neural substrates where this interaction might occur. Most notably, the hippocampal

excitatory circuit receives tonic excitatory cholinergic input from the medial septum and the diagonal band of Broca and glutamate, acting on NMDA receptors located on inhibitory GABAergic interneurons within the septum, inhibits the activity of cholinergic neurons that project to the hippocampus (11). Consistent with this localization, behavioral and electrophysiological studies have shown that memantine increases cholinergic signaling and excitability in mouse hippocampus and that this action is blocked by the muscarinic antagonist scopolamine (7). Moreover, it was shown that galantamine induces cholinergic activation via acetylcholinesterase inhibition and allosteric stimulation of nicotinic receptors (12). Therefore, the memory potentiation documented in the present study might be due to a cholinergic activation resulting from the concomitant treatment with memantine and galantamine, acting through both nicotinic and muscarinic pathways.

Interestingly, a clinical study on AD patients showed that galantamine treatment increased absolute glutamate levels in the hippocampus and this was associated to an overall increase in cognitive performances (13). Moreover, in rat hippocampal slices, galantamine potentiates the excitatory postsynaptic current amplitude binding to nicotinic receptors and acting as an allosteric potentiating ligand. In this way, it sensitizes the receptor to its activation by acetylcholine, which in turn stimulates glutamatergic release (14). Thus, since neurotransmission within the hippocampus involves the interaction of both glutamatergic and cholinergic signal transduction mechanisms, galantamine may act to increase either cholinergic or glutamatergic neurotransmission. An overall enhancement of the glutamatergic signaling induced by galantamine could then contribute to its beneficial effects: the increase in glutamate release stimulates post-synaptic NMDA receptors and produces positive effects on learning and memory. It is known, however, that an excess in glutamate levels and signaling leads to detrimental effects on neurons (15): we can hypothesize that co-administration of memantine counteracts the negative effects of excessive glutamate release—partially due to galantamine—mainly acting on the extrasynaptic receptors.

Other neurotransmitter systems could be involved in the actions of galantamine and memantine action. In particular, galantamine's activity on nicotinic receptors located on non-cholinergic neurons stimulates the release of other neurotransmitters such as dopamine, noradrenaline, and GABA in several brain regions including the prefrontal cortex, striatum, nucleus accumbens, and hippocampus (for a review, see 16). On the other hand, memantine administration results in significant increases in extracellular dopamine, noradrenaline, and their metabolites in the cortical regions and in dopamine reduc-

tion in rat hippocampus (17). The influence of galantamine and memantine on the release of dopamine and noradrenaline may contribute to the beneficial effects of their co-administration on the non-cognitive symptoms of dementia that are usually correlated to these neurotransmitters (16).

Altogether, these neurochemical alterations in key cognitive areas of the brain might lead to complex implications on learning and memory mechanisms and contribute to the beneficial activity of the combination of the two compounds.

In light of the multifactorial nature of cognitive and neurodegenerative disorders, the synergistic effect of low and ineffective doses of memantine and galantamine may provide a starting point for new treatment strategies for cognitive impairment. Notably, this study could also suggest the use of reduced doses of the two drugs with lower side effects laying the bases for a multitargeted approach to AD that could overcome some of the major limitations of the currently available drugs given in combination, in terms of increased efficacy, reduced side effects, and improved compliance.

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