Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmacology 571 (2007) 174–179
www.elsevier.com/locate/ejphar

# Memantine and recognition memory: Possible facilitation of its behavioral effects by the nitric oxide (NO) donor molsidomine

Nikolaos Pitsikas *, Nikolaos Sakellaridis

*Department of Pharmacology, School of Medicine, University of Thessaly, 22, Papakiriazi str., 412-22 Larissa, Greece*

Received 7 February 2007; received in revised form 4 June 2007; accepted 7 June 2007
Available online 27 June 2007

**Abstract**

The effects of the non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist memantine on recognition memory were investigated in the rat by using the object recognition task. In addition, a possible interaction between memantine and the nitric oxide (NO) donor molsidomine in antagonizing extinction of recognition memory was also evaluated utilizing the same behavioral procedure. In a first dose-response study, post-training administration of memantine (10 and 20, but not 3 mg/kg) antagonized recognition memory deficits in the rat, suggesting that memantine modulates storage and/or retrieval of information. In a subsequent study, combination of sub-threshold doses of memantine (3 mg/kg) and the NO donor molsidomine (1 mg/kg) counteracted delay-dependent impairments in the same task. Neither memantine (3 mg/kg) nor molsidomine (1 mg/kg) alone reduced object recognition performance deficits. The present findings indicate a) that memantine is involved in recognition memory and b) support a functional interaction between memantine and molsidomine on recognition memory mechanisms.


*Keywords:* NMDA; Nitric oxide; Memantine; Molsidomine; Recognition memory; (Rat)

## 1. Introduction

The *N*-methyl-D-aspartate (NMDA) receptor antagonist memantine has been approved for the treatment of severe Alzheimer Disease (Reisberg et al., 2003; Scarpini et al., 2003). Memantine is a non-competitive NMDA receptor antagonist of moderate affinity that could decrease pathological activation of NMDA receptors without affecting physiological NMDA receptor activity (Scarpini et al., 2003). This compound has been shown to improve rodents' performance in tasks usually assessing spatial memory in different amnestic animal models (Barnes et al., 1996; Zaiaczkowski et al., 1996; Wenk et al., 1997; Parsons et al., 1999; Zoladz et al., 2006).

Nitric oxide (NO) is considered a retrograde intracellular messenger in the brain (Garthwaite, 1991). Its implication in cognition is well documented (Prast and Philippu, 2001). Behavioral investigations have demonstrated that blockade of NO synthase (NOS) by different NOS inhibitors impairs animals' performance in various learning and memory paradigms (Fin et al., 1995; Meyer et al., 1998; Pitsikas et al., 2003). These deficits could be counteracted by diverse NO donors (Fin et al., 1995; Meyer et al., 1998; Pitsikas et al., 2003). Among NO donors, molsidomine has a high bioavailability, a long-lasting duration of action (Boger et al., 1994) likely crosses the blood-brain barrier (Maccario et al., 1997) and increases its permeability (Mayhan, 2000). It has been observed that molsidomine lacks overt side-effects at doses displaying an antiamnestic action in recognition memory tasks in the rat (Pitsikas et al., 2001, 2002, 2003, 2005).

Recognition memory stems from a series of neural processes by which a subject is aware that a stimulus has been previously experienced, recognition being the behavioral outcome of these processes (Steckler et al., 1998). The aim of the present study was first to investigate the role of memantine on recognition memory.

Although memantines' effects on memory seem to be linked to the NMDA receptor, up to now, no study has addressed if these effects might involve the nitrergic system. Several lines of evidence indicate a relationship between NO and the NMDA receptor: NO synthesis is stimulated by $Ca^{2+}$-influx which is induced by activation of the NMDA receptor (Garthwaite,

* Corresponding author. Tel.: +30 2410 565268; fax: +30 2410 565236.
*E-mail address:* npitsikas@med.uth.gr (N. Pitsikas).

0014-2999/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2007.06.019

1991). The largest amount of neuronal NOS is localized in proximity of post-synaptic specializations, where a high density of NMDA receptors is found (Brenman and Bredt, 1997). The role exerted by NO on synaptic plasticity (induction of long-term potentiation) occurs in close association with the glutamatergic system (Brenman and Bredt, 1997). Finally, it has been observed that low concentrations of the NO donor SNAP inhibits glutamate release in hippocampus, whereas high concentrations of this NO donor produced opposite effects (Segieth et al., 1995). Therefore, in a second set of experiments we sought to be of interest to investigate a possible interaction of molsidomine with the responses elicited by memantine in a memory task.

For these studies, the object recognition test, a non-rewarded paradigm, based on the spontaneous exploratory behavior of rats, which reflects non-spatial working memory, was selected (Ennaceur and Delacour, 1988). The choice of this task was sought to be of interest, since to our knowledge, the effects of memantine on cognition were not assessed by a recognition memory procedure.

## 2. Materials and methods

Procedures involving animals and their care were conducted in conformity with the international guidelines, in compliance with National and International laws and policies (EEC Council Directive 86/609, JL 358, 1, December 12, 1987; *NIH Guide for Care and Use of Laboratory Animals*, NIH publication no. 80–23, revised 1996).

### 2.1. Animals

Male (3-month-old) Wistar rats (Hellenic Pasteur Institute, Athens, Greece), weighing 250–300 g, were used in this study. The animals were housed in Makrolon cages (45 cm long × 35 cm high × 20 cm wide), three per cage, in a regulated environment (21 ± 1 °C; 50–55% relative humidity; 12-light/12-dark cycle, lights on at 07:00 h), with free access to food and water. Experiments were conducted in the room housing exclusively these animals, and took place between 09:00 and 13:00 h. Behavioral observations and evaluations were performed by experimenters who were unaware of the pharmacological treatment.

### 2.2. Object recognition test

#### 2.2.1. Apparatus

The test apparatus consisted of an open box made of plexiglas (80 cm long × 50 cm high × 60 cm wide), which was illuminated by a 60-W lamp suspended 60 cm above the box. In the different parts of the apparatus the light intensity was equal. The objects to be discriminated were in three different shapes: cubes, pyramids and cylinders 7 cm high; they could not be displaced by rats. The cubes were from metal, the pyramids were from glass and the cylinders were plastic. These objects had no genuine significance for rats and had never been associated with reinforcement.

#### 2.2.2. Procedure

The object recognition test was performed as described elsewhere (Ennaceur and Delacour, 1988). In the week preceding testing, the animals were handled twice daily. On the day before testing, they were allowed to explore the apparatus for 2 min, while on the testing day 1, a single 2-min "sample" trial (T1) was given. During T1, two identical samples (objects) (e.g., 2 plastic cylinders) were placed in two opposite corners of the apparatus 10 cm from the sidewall. A rat was placed in the middle of the apparatus and was left to explore these two identical objects. After T1, the rat was put back in its home cage and an intertrial interval (ITI) of 24 h was given. On the testing day 2, a single 2-min "choice" trial (T2) was performed. During T2, a new object (*N*) different from the familiar object either as texture or as shape (i.e., a metallic cube) replaced one of the samples presented in T1; then, the rats were exposed again to two objects: a copy of the familiar (*F*) and the new one *N*. All combinations and locations of objects were used in a balanced manner to reduce potential biases due to preferences for particular locations or objects. To avoid the presence of olfactory trails, the apparatus and the objects after each trial were thoroughly cleaned.

Exploration was defined as follows: directing the nose toward the object at a distance of no more than 2 cm and/or touching the object with the nose. Turning around or sitting on the object was not considered as exploratory behavior. The times spent by rats in exploring each object during T1 and T2 were recorded manually by using a stopwatch. From this measure, a series of variables was then calculated: the total time spent in exploring the two identical objects in T1, and that spent in exploring the two different objects, *F* and *N* in T2. To evaluate whether or not within each group, animals had manifested a preference either for an object or for a location, the exploration times were analyzed according to the nature of objects and locations of the apparatus. The discrimination between *F* and *N* during T2 was measured by comparing the time spent in exploring the familiar sample with that spent in exploring the new object. As this time may be biased by differences in overall levels of exploration, a discrimination index (*D*) was then calculated; $D=N-F/N+F$. *D* is the discrimination ratio and represents the difference in exploration time expressed as a proportion of the total time spent exploring the two objects in T2 (Cavoy and Delacour, 1993). In addition, motor activity of each animal expressed as total number of steps during each trial was also recorded.

### 2.3. Drugs

Memantine (1-amino-3,5-dimethyl-adamantane; Sigma, St. Louis, MO, USA) and molsidomine [(*N*-[ethoxycarbonyl]-3-[4-morpholinosydnomine] (Sigma Tau, Milan, Italy) were dissolved in saline (NaCl 0.9%) and injected intraperitoneally (i.p.). The dose of molsidomine (1 mg/kg) was chosen on the basis of a preliminary study in which it was found ineffective against time-related retention deficits in the object recognition task and did not produce adverse side-effects (unpublished our observations). Control animals received the vehicle (NaCl 0.9%).