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Effects of molsidomine on scopolamine-induced amnesia and hypermotility in the rat

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Abstract

Nitric oxide (NO) is hypothesized to be a novel intracellular messenger in the central nervous system. Recently, NO involvement in learning and memory processes has been proposed. Compounds that inhibit nitric oxide synthase, the key synthesizing enzyme, may block cognition, while NO donors may facilitate it. The aim of this study was to assess in the rat the effects of the NO donor molsidomine (2 and 4 mg/kg, i.p.) on memory deficits caused by scopolamine. For this purpose, the object recognition task and the step-through passive avoidance procedure were chosen. In addition, the effects of molsidomine in antagonizing the scopolamine-induced hypermotility were also examined. Scopolamine at 0.2 mg/kg (object recognition) and 0.75 mg/kg (passive avoidance) disrupted acquisition in both the tasks and induced locomotor hyperactivity at the dose of 0.2 mg/kg. Molsidomine at either dose reversed the scopolamine-induced deficits in the object recognition paradigm but did not counteract the hypermotility and the deficits occurred in the passive avoidance test. These results suggest that to some extent, the NO donor molsidomine is involved in memory processing.

Keywords: Nitric oxide (NO); Scopolamine; Molsidomine; Memory; Motility; (Rat)

1. Introduction

Nitric oxide (NO), a soluble, short lived and freely diffusible gas, has been proposed to act as a novel retrograde intracellular messenger in the brain since it enhances presynaptic release of glutamate via guanylyl cyclase mediated mechanisms (Garthwaite, 1991; Moncada et al., 1991; Schuman and Madison, 1994). NO is synthesized from arginine by a Ca^{2+}/calmodulin-dependent enzyme, NO synthase (NOS) (Garthwaite, 1991).

Reportedly, NO is involved in the mechanisms of synaptic plasticity, including long-term potentiation in the hippocampus (O'Dell et al., 1991; Haley et al., 1992; Bannerman et al., 1994). Intracellular application of a NO scavenger or use of a UV-sensitive NO donor elicits long-term potentiation (Arancio et al., 1996). The implication of NO in learning and memory formation has also been proposed (Ingram et al., 1996). Several behavioral investigations, performed in different rodent models, have demonstrated that compounds which block NOS inhibit learning (Madison and Schuman, 1991; Chapman et al., 1992; Bohme et al., 1993; Yamada et al., 1996; Prickaerts et al., 1997; Blokland et al., 1998; Meyer et al., 1998), while other studies have not supported this proposition (Okere et al., 1995; Tobin et al., 1995; Holscher et al., 1996; Knepper and Kurylo, 1998). In addition, recent studies have demonstrated that the NO donors, *S*-nitroso-*N*-acetylpenicillamine, sodium nitroprusside and molsidomine, attenuated learning deficits induced by the NOS inhibitors *N*-nitroarginine (NOArg) and 7-nitroindazole (Fin et al., 1995; Huang and Lee, 1995; Meyer et al., 1998).

A major problem when using NO inhibitors or donors relates to their effects on blood pressure (Varner and Beckman, 1995) since NOS inhibitors cause hypertension, whereas NO donors hypotension. It is difficult, therefore,

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to quantify how these cardiovascular effects may affect cognition.

Among NO donors, molsidomine is fairly well absorbed, has a long-lasting duration of action (Boger et al., 1994) and, at doses displaying an antiamnestic action, it lacks overt side-effects (Meyer et al., 1998). To our knowledge, there are no specific pharmacokinetic studies on the penetration of molsidomine through the blood–brain barrier. However, though inferentially, such a possibility is supported by studies on the neuroendocrine and orexigenic effects of this compound administered systemically (Maccario et al., 1997; Rigamonti et al., 2001).

It is well known that the cholinergic system is critically involved in learning and memory formation (Bartus et al., 1982) and several observations have led to propose a functional interaction between NO and this system (Prast and Philippu, 1992; Kilbinger, 1996; Kopf and Baratti, 1996).

Therefore, we decided to investigate the efficacy of molsidomine in antagonizing cholinergic hypofunction produced by treatment with scopolamine in two memory paradigms that involve different memory mechanisms: the object recognition task (Ennaceur and Delacour, 1988) and the step-through passive avoidance procedure (King and Glasser, 1970). In addition, the ability of molsidomine to counteract scopolamine-induced hypermotility was also assessed in a locomotor activity test.

2. Materials and methods

Procedures involving animals and their care were conducted in conformity with the institutional guidelines, in compliance with national and international laws and policies.

2.1. Subjects

Different populations of male (2-month-old) CD-COBS rats (Charles River, Calco, Italy), weighing 250–300 g were used in this study. The animals were housed in Makrolon cages ($35 \times 45 \times 20$ cm), four 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 were performed by an experimenter who was unaware of the pharmacological treatment.

2.2. Drugs

Scopolamine HBr and methylscopolamine HBr (Sigma, St. Louis, MO, U.S.A.) were dissolved in saline and injected s.c. Molsidomine (Sigma Tau, Milan, Italy) was dissolved in saline and administered i.p. Doses of compounds are expressed as bases. Control animals received the vehicle (NaCl, 0.9%).

2.3. Object recognition task experiments

2.3.1. Apparatus

The test apparatus consisted of a dark open box made of Plexiglas ($80 \times 50 \times 60$ cm), 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 (in triplicate) were made of glass, plastic, or metal, were in three different shapes: cubes, pyramids and cylinders 7-cm high; they could not be displaced by rats. In addition, these objects had no genuine significance for rats and had never been associated with a reinforcement.

2.3.2. 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, the animals were allowed to explore the apparatus for 2 min, while on the testing day, a session of two 2-min trials was given. During the "sample" trial (T1), two identical samples (objects) were placed in two opposite corners of the apparatus. A rat was placed in the middle of 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 was given. Subsequently, the "choice" trial (T2), was performed. During T2, a new object (N) replaced one of the samples presented in T1, thence, the rats were then exposed again to two objects: 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, familiar and novel in T2. The discrimination between the familiar and the novel object 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 discrimi-

nation ratio and represents the difference in exploration time expressed as a proportion of the total time spent exploring the two objects in T2.

2.3.3. Effects of scopolamine and methylscopolamine on object recognition memory tested at different intertrial intervals

The aim of this study was to investigate at which intertrial interval (1-min, 15-min, or 60-min) scopolamine affected the object recognition memory. In addition, the effects of the peripheral muscarinic receptor antagonist methylscopolamine on the object recognition were also assessed by using a 60-min delay procedure.

2.3.3.1. Experimental design and drug treatment. Animals were randomly divided into seven experimental groups (10 rats per group) as follows: vehicle-1 min; vehicle-15 min; vehicle-60 min; scopolamine 0.2 mg/kg-1 min; scopolamine 0.2 mg/kg-15 min; scopolamine 0.2 mg/kg-60 min and methylscopolamine 0.2 mg/kg-60 min. The dose of scopolamine was selected based on a study where the drug disrupted performance in the object recognition test at an intertrial interval of 60 min (Bartolini et al., 1996).

Control rats were given s.c. the vehicle, 60 min before starting T1. Scopolamine and methylscopolamine were injected 60 min before T1.

2.3.4. Effects of molsidomine in antagonizing scopolamine-induced amnesia in the object recognition task

For this study, the 60-min intertrial interval has been selected since scopolamine at this delay condition impaired object recognition in the young rat (Bartolini et al., 1996).

2.3.4.1. Experimental design and drug treatment. Animals were randomly divided into six experimental groups (10 rats per group) as follows: vehicle plus vehicle; vehicle plus molsidomine 2 mg/kg; vehicle plus molsidomine 4 mg/kg; vehicle plus scopolamine 0.2 mg/kg; scopolamine 0.2 mg/kg plus molsidomine 2 mg/kg; and scopolamine 0.2 mg/kg plus molsidomine 4 mg/kg. Doses of molsidomine were chosen on the basis of a study in which they were effective against learning impairments and did not produce adverse side-effects (Meyer et al., 1998).

Control rats were given i.p. and s.c. the vehicle, 60 min before starting T1. Scopolamine and molsidomine were coadministered 60 min before T1.

2.4. Step-through passive avoidance experiment

2.4.1. Apparatus

The apparatus was composed of a large compartment with a grid floor ($50 \times 50 \times 50$ cm) made of Plexiglas connected to an illuminated platform. The platform was separated from the compartment by a guillotine door. Electric shocks were delivered to the grid floor by an isolated stimulator.

2.4.2. Procedure

The procedure described by King and Glasser (1970) was used. During the first day (training session), each rat was gently placed on the illuminated platform, and 10 s later, the guillotine door was opened. As soon as the rat has moved into the dark chamber and the door has been shut, a 1.6-mA footshock was applied for 1 s. Thereafter, the rat was immediately removed from the apparatus and returned to the home cage.

During the second day, the retention trial was performed. Each rat was placed in the lighted compartment and the step-through latency was recorded. This is the time the rat remained on the illuminated platform. The test was stopped as soon as the rat entered the dark chamber, or remained on the illuminated platform for 180 s.

2.4.3. Experimental design and drug treatment

Animals were randomly divided into six experimental groups (10 rats per group) as follows: vehicle plus vehicle; vehicle plus molsidomine 2 mg/kg; vehicle plus molsidomine 4 mg/kg; vehicle plus scopolamine 0.75 mg/kg; scopolamine 0.75 mg/kg plus molsidomine 2 mg/kg; and scopolamine 0.75 mg/kg plus molsidomine 4 mg/kg. Doses of molsidomine were chosen on the basis of a study in which they were effective against learning impairments and did not produce adverse side-effects (Meyer et al., 1998). The dose of scopolamine was selected on the basis of our prior experience in this behavioral procedure (Brambilla et al., 1993; Pitsikas and Borsini, 1997).

Control rats were treated with the vehicle, 60 and 30 min, i.p. and s.c., respectively, before starting the training session. Scopolamine was injected 30 min before starting the training session; molsidomine was administered 60 min before starting the training session.

2.5. Motor activity experiment

2.5.1. Method

Spontaneous motor activity was assessed in an activity cage (Ugo Basile, Varese, Italy). The cage was fitted with two parallel horizontal and vertical infrared beams, respectively, 2 and 6 cm from the floor.

2.5.2. Procedure

Each rat was placed in the apparatus for a 30-min habituation period. Thereafter, horizontal and vertical motor activity was monitored for 10 min (Braida et al., 2000).

2.5.3. Experimental design and drug treatment

Animals were randomly divided into six experimental groups (eight rats per group) as follows: vehicle plus vehicle; vehicle plus molsidomine 2 mg/kg; vehicle plus

molsidomine 4 mg/kg; vehicle plus scopolamine 0.2 mg/kg; scopolamine 0.2 mg/kg plus molsidomine 2 mg/kg; and scopolamine 0.2 mg/kg plus molsidomine 4 mg/kg. Doses of molsidomine were chosen on the basis of a study in which they were effective against learning impairments and did not produce adverse side-effects (Meyer et al., 1998).

Control rats were given i.p. and s.c. the vehicle, 60 min before testing. Scopolamine and molsidomine were administered 60 min before testing.

2.6. Statistical analysis

2.6.1. Object recognition experiment

Data are calculated as medians and interquartile ranges. Comparisons of total exploration times throughout trials (T1 vs. T2) for each group separately were made by the nonparametric Mann–Whitney test. When comparing the total exploration times of the different groups within T1 and T2, the nonparametric Kruskal–Wallis analysis of variance followed by the Dunn's post-hoc test was used. When comparing within each group exploration times of the familiar and the novel object during T2, the nonparametric Mann–Whitney test was applied. Discrimination index *D* data were calculated by the Kruskal–Wallis nonparametric test followed by the Dunn's post-hoc test.

2.6.2. Step-through passive avoidance experiment

Data are calculated as medians and interquartile ranges. Pre- and post-shock latencies were evaluated by the Kruskal–Wallis nonparametric test followed by the Dunn's post-hoc test.

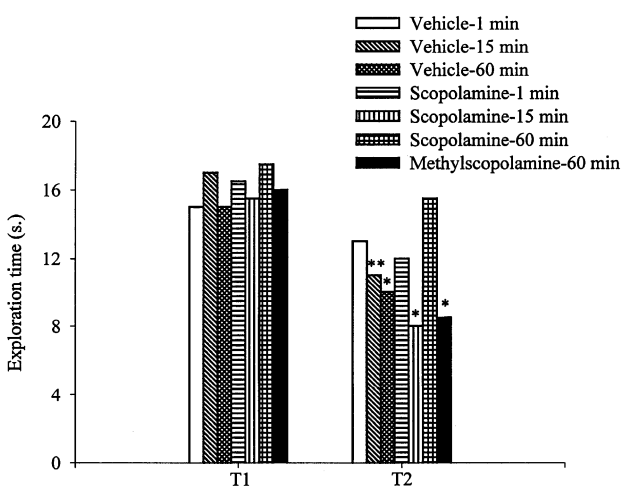

Fig. 1. Total exploration time displayed by different groups of rats in the object recognition test. Vehicle and scopolamine were injected subcutaneously, 60 min before starting T1. The intertrial interval was 1, 15 or 60 min. Results are expressed as medians. $^{**}P < 0.01$, $^{*}P < 0.05$ vs. the same groups of rats within T1. See text for details. The same description applies to Figs. 2 and 3.

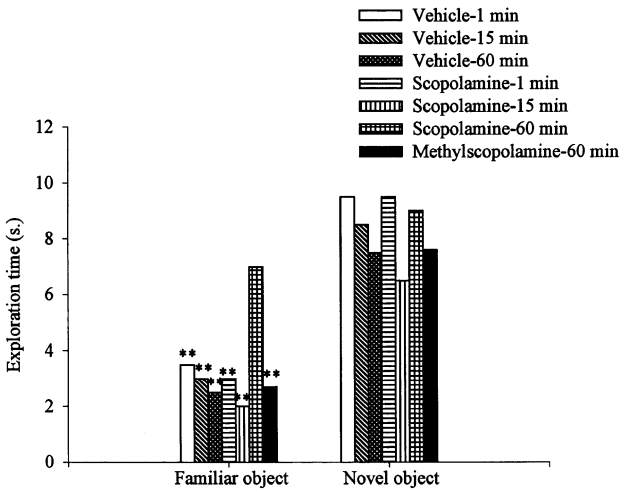

Fig. 2. Comparison of the exploration time of the familiar vs. the novel object during T2 displayed by different groups of rats in the object recognition test. Results are expressed as medians. $^{**}P < 0.01$ vs. the novel object.

2.6.3. Motor activity experiment

Results are expressed as mean ± S.E.M. Differences between groups were evaluated by the one-way ANOVA test followed by the Duncan's post-hoc test.

All statistical analysis were carried out by using the SIGMA Stat Program, Version 2.0, Jandel, U.S.A.

3. Results

3.1. Effects of scopolamine and methylscopolamine on object recognition memory tested at different intertrial intervals

General exploratory activity of vehicle-1-min, scopolamine-1-min, and scopolamine-60-min-treated rats did not

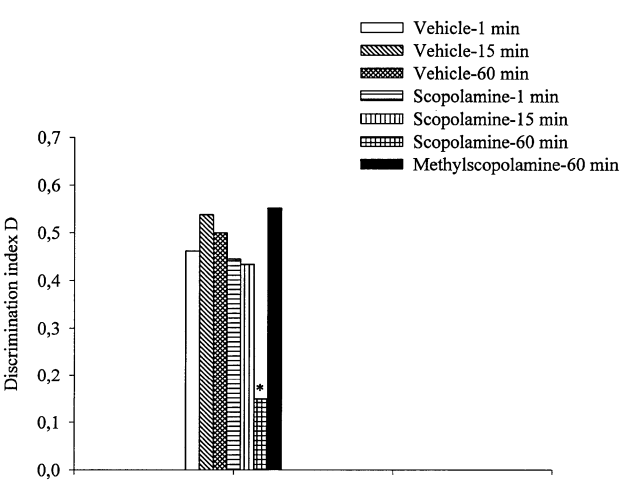

Fig. 3. Discrimination index *D* performance expressed by different groups of rats during T2 in the object recognition test. Results are expressed as medians. $^{*}P < 0.05$ vs. all the other groups.

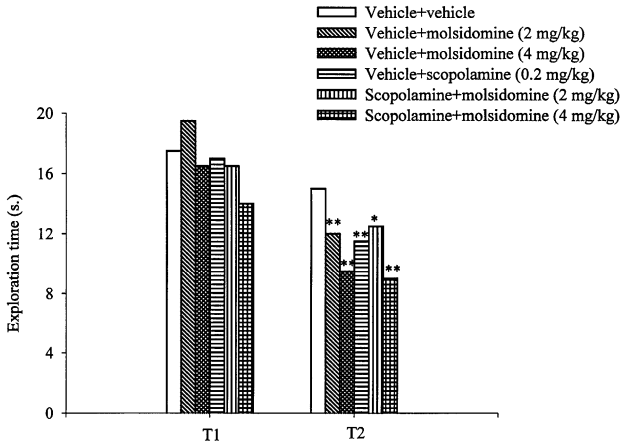

Fig. 4. Total exploration time displayed by different groups of rats in the object recognition test. Scopolamine and molsidomine were injected subcutaneously and intraperitoneally, respectively, 60 min before starting T1. The intertrial interval was 60 min. Results are expressed as medians. $^{**}P < 0.01$, $^{*}P < 0.05$ vs. the same groups of rats within T1. See text for details. The same description applies to Figs. 5 and 6.

change throughout trials. Comparing with the respective exploratory activity exhibited in T1, a reduction of it in T2 was observed in the vehicle-15-min, vehicle-60-min, scopolamine-15-min and methylscopolamine-60-min groups; [$P < 0.01$; $P < 0.05$; $P < 0.05$; and $P < 0.05$, respectively (Fig. 1)]. Within T1 and T2, no differences in total exploration time were seen among the different groups [$H(6) = 2.9$, $P = 0.82$, not significant; $H(6) = 7.4$, $P = 0.28$, not significant, respectively, for T1 and T2 (Fig. 1)].

During T2, all animals, except the scopolamine-60-min-treated rats, explored significantly more the novel than the familiar object ($P < 0.01$). Scopolamine-60-min-treated rats explored similarly either objects [$P = 0.1$, not significant (Fig. 2)].

Index D data revealed that all rats, except those treated with scopolamine (60-min delay condition), discriminated

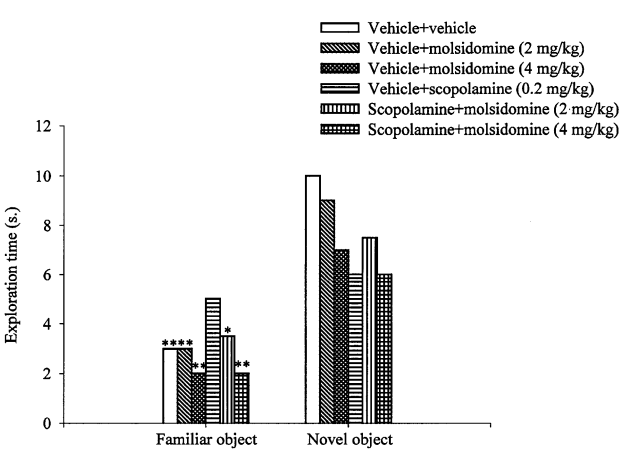

Fig. 5. Comparison of the exploration time of the familiar vs. the novel object during T2 displayed by different groups of rats in the object recognition test. Results are expressed as medians. $^{**}P < 0.01$, $^{*}P < 0.05$ vs. the novel object.

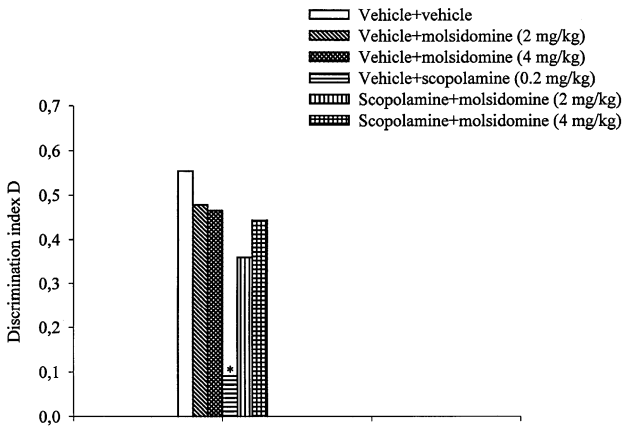

Fig. 6. Discrimination index D performance expressed by different groups of rats during T2 in the object recognition test. Results are expressed as medians. $^{*}P < 0.05$ vs. all the other groups.

significantly better the new object than the familiar one; [$H(6) = 23.1$, $P < 0.01$; Dunn's post-hoc test, $P < 0.05$ vs. scopolamine-60-min group (Fig. 3)].

3.2. Effects of molsidomine in antagonizing scopolamine-induced amnesia in the object recognition task

General exploratory activity was significantly reduced throughout trials in all groups, except the vehicle plus vehicle-treated rats; [for vehicle plus molsidomine 2 mg/kg; vehicle plus molsidomine 4 mg/kg; vehicle plus scopolamine and scopolamine plus molsidomine 4 mg/kg, $P < 0.01$; for scopolamine plus molsidomine 2 mg/kg, $P < 0.05$ (Fig. 4)]. Within T1 and T2, no differences in total exploration time were seen among the different groups [$H(5) = 4.9$, $P = 0.43$, not significant; $H(5) = 10.8$, $P = 0.06$, not significant, respectively, for T1 and T2 (Fig. 4)].

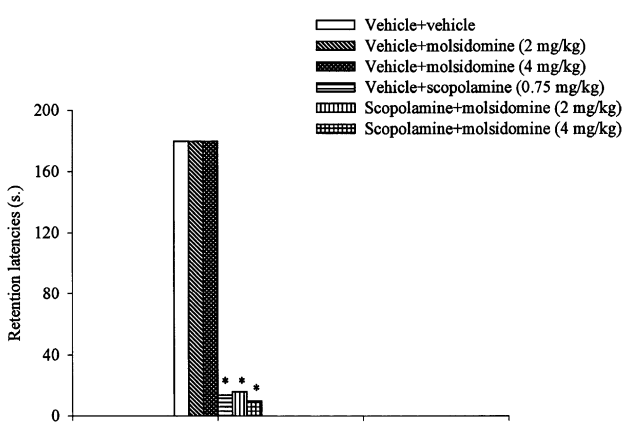

Fig. 7. Retention latencies to enter the dark chamber in the step-through passive avoidance test displayed by different groups of rats. Scopolamine and molsidomine were injected subcutaneously and intraperitoneally 30 and 60 min, respectively, before starting the training session. Results are expressed as medians. $^{*}P < 0.05$ vs. the respective control groups. See text for details.

During T2, vehicle plus vehicle, vehicle plus molsidomine 2 mg/kg, and vehicle plus molsidomine 4 mg/kg-treated rats explored significantly more the novel than the familiar object; [$P < 0.01$ (Fig. 5)]. Animals treated with vehicle plus scopolamine did not explore more the novel than the familiar; [$P = 0.16$, not significant (Fig. 5)]. In the scopolamine-treated rats, molsidomine (2 and 4 mg/kg) significantly restored the loss of object recognition; [$P < 0.05$; $P < 0.01$, respectively (Fig. 5)].

Index D data revealed that all rats, except those treated with vehicle plus scopolamine, discriminated significantly better the new object than the familiar one; [$H(5) = 17.4$, $P < 0.01$; Dunn's post-hoc test, $P < 0.05$ vs. scopolamine-60-min group (Fig. 6)].

3.3. Effects of molsidomine in antagonizing scopolamine-induced amnesia in the step-through passive avoidance task

Pre-shock latencies were not different among all experimental groups [$H(5) = 9.6$, $P = 0.09$, not significant, data not shown]. All scopolamine-treated animals displayed shorter retention latencies compared to the control populations [$H(5) = 34.5$, $P < 0.01$]. Treatment with molsidomine failed to reverse scopolamine-induced amnesia in this test (Fig. 7).

3.4. Effects of molsidomine in antagonizing scopolamine-induced hypermotility in a motor activity task

Differences either in horizontal or vertical motor activity among different groups were observed: [$F(5,42) = 17.8$, $P < 0.01$, for horizontal activity; $F(5,42) = 12.1$, $P < 0.01$, for vertical activity, Fig. 8]. Post-hoc comparisons have demonstrated that treatment with scopolamine resulted in a significant increase of both horizontal and vertical activity: [scopolamine plus vehicle vs. vehicle plus vehicle, $P < 0.05$; scopolamine plus molsidomine 2 mg/kg vs. vehicle plus molsidomine 2 mg/kg, $P < 0.05$ and scopolamine plus 4 mg/kg vs. vehicle plus molsidomine 4 mg/kg, $P < 0.05$]. Molsidomine at any dose did not antagonize this scopolamine-induced hypermotility. No differences between vehicle plus vehicle and molsidomine-treated rats were recorded.

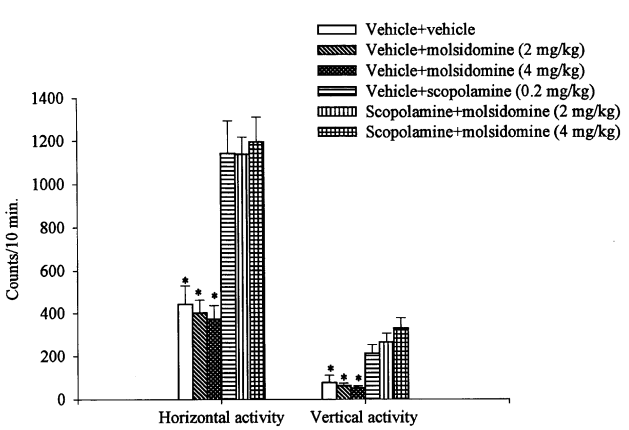

Fig. 8. Cumulative scores (counts/10 min) in the motor activity test displayed by different groups of rats. Scopolamine and molsidomine were injected subcutaneously and intraperitoneally, respectively, 60 min before starting testing. Results are expressed as mean ± S.E.M. * $P < 0.05$ vs. the respective control groups.

4. Discussion

Object recognition is a form of nonspatial working memory, usually investigated in monkeys and rats by rewarded matching- or nonmatching-to-sample tasks (Bartolini et al., 1996). The object recognition paradigm used in our study can be regarded as a spontaneous nonmatching-to-sample task (Steckler et al., 1998). Prior findings suggest that object recognition may be considered as a form of episodic memory that lasts at least 60 min in the young rat, a situation in which scopolamine reduced it (Bartolini et al., 1996). However, since intertrial delays were not systematically varied in that study, the timing of scopolamine-induced performance deficit in this working memory paradigm had to be established.

There is experimental evidence that peripherally administered scopolamine may not primarily and/or selectively affect learning performance and memory processes but rather influence sensory/attentional processes (Blokland, 1996). It has also been suggested that scopolamine-induced delay-independent deficits may reflect an impairment in attentional and/or sensory responding, while delay-dependent deficits are interpreted as resembling forgetting (Chudasama and Muir, 1997).

Our results demonstrate that recognition memory abilities in the young vehicle-treated rat remained intact at any delay condition tested (1-min, 15-min and 60-min), whereas object recognition was affected by scopolamine only at a 60-min delay. This delay-dependent deficit cannot be ascribed to an overall impairment of exploratory behavior since the total exploration times of these rats did not vary significantly throughout trials. In addition, methylscopolamine, a muscarinic receptor antagonist, rather impermeable to the blood–brain barrier, did not impair object recognition when given at the intertrial condition of 60 min. Thus, the scopolamine-induced working memory decay seems to be a centrally mediated event. In addition, attentional or sensorymotor factors would not interfere with the memory delay-dependent decline observed in our study.

Vehicle and molsidomine-treated rats acquired similarly well the object recognition paradigm which was disrupted by scopolamine, and molsidomine, at either dose used successfully antagonized the scopolamine-induced impairment. In this context, molsidomine restored object recognition by reducing the time spent in exploring the familiar object. During T1, the exploration levels of rats treated

either with scopolamine or/and molsidomine were similar, whereas during T2, all drug-treated rats explored significantly less the two objects (familiar and novel).

Object recognition task is based on spontaneous exploratory activity and, since the latter may be implicated in anxiogenic or anxiolytic processes, the compounds we used might have acted on animal performance through anxiolytic or anxiogenic mechanisms. In this context, contrasting effects of scopolamine have been reported on anxiety. It has been claimed that scopolamine is anxiogenic (Smythe et al., 1996), not involved in anxiety processes (Rodgers et al., 1996) or exerting an anxiolytic effect when given together with the benzodiazepines (Belotti et al., 1998). The NO-ergic system has been implicated in the mechanisms of anxiety and reciprocally, NOS inhibitors were reported to have anxiolytic properties in a variety of behavioral tasks (Faria et al., 1997; Dunn et al., 1998). Though the role of molsidomine per se in anxiety has never been investigated, based on the reported findings, it seems unlikely that the effects observed in our study could be accounted by the anxiogenic/anxiolytic properties of the compounds used.

Reportedly, NO donors possess hypotensive properties (Varner and Beckman, 1995), and in our study, although the doses of molsidomine used were very low and claimed to be devoid of side-effects (Meyer et al., 1998), the possibility of a nonspecific effect of the compound could not be completely ruled out. However, all molsidomine-treated rats irrespective whether combined with or not with scopolamine acquired well the task.

Similar to our findings, in a different memory paradigm (14-unit Stone maze vs. object recognition) and by using a different amnestic agent (7-nitroindazole vs. scopolamine), molsidomine also proved capable to reverse memory deficits in the rat (Meyer et al., 1998).

Passive avoidance behavior evidenced a good retention in the control rats and a poor performance in all the scopolamine-treated rats. Molsidomine, at any dose, was unable to counteract the scopolamine-induced deficit and also did not influence the performance of controls.

There is no sound reason for molsidomine's failure to antagonize the scopolamine-induced deficits in this test. Reportedly, pharmacological treatment given before the noxious stimulus may affect different parameters not related with cognition (i.e. pain perception, motor activity) (Sarter et al., 1992). However, along this line, molsidomine induced-antinociception can be excluded (Fidecka and Lalewicz, 1997). The antinociceptive effect of molsidomine occurred at high doses (150 and 300 mg/kg) and not at the low doses of our study and, moreover, molsidomine did not affect the performance of control rats.

Similarly, an effect of scopolamine related to its hyperactivity properties would be unlikely. In fact, pre-shock latencies were not different among the experimental groups and scopolamine was injected only once, 30 min before starting the acquisition trial.

Results obtained in different experimental settings may differ for several reasons, among which the different doses of the compounds used. For instance, we cannot rule out the possibility that the apparent failure of molsidomine to antagonize scopolamine-induced amnesia in the passive avoidance task might be related to the higher dose of scopolamine used in this paradigm. In this context, the use of a higher dose of molsidomine and the evaluation of its effect would have been explanatory.

Scopolamine-induced hypermotility was disclosed by a specific motor activity task. This represents a behavioral effect caused by many anticholinergic drugs, which do not involve cognitive performance (Bushnell, 1987), and in our study was not antagonized by molsidomine.

The dual ability of molsidomine to counteract scopolamine-induced cognitive and noncognitive effects reinforces the idea that the latter have different underlying mechanisms.

There is scant evidence for other behavioral effects of molsidomine and the underlying mechanisms of action (Meyer et al., 1998).

In summary, our study demonstrates that low doses of molsidomine antagonized scopolamine-induced object recognition memory impairments, bespeaking the involvement of the NO-ergic system in the cholinergic modulation of memory. Supporting this proposition, a variety of NO donors have been shown capable to potentiate basal acetylcholine release from different brain areas (Prast and Philippu, 1992; Kilibinger, 1996; Kar et al., 1998).

Our data, thus, reinforce the idea that molsidomine may be a useful adjunct to the treatment of memory impairments, though further preclinical investigations and the use of genuine models of memory deficits, e.g. elderly rats, are mandatory for assessing its therapeutic potential.

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