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Molsidomine attenuates N^{ω}-nitro-L-argininemethylester-induced deficits in a memory task in the rat

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Abstract

The present study was designed to investigate the role of nitric oxide (NO) on recognition memory in the rat. For this purpose, the effects on memory exerted by post-training administration of the NO synthase (NOS) inhibitor N^{ω}-nitro-L-argininemethylester (L-NAME) and the NO donor molsidomine were assessed by using the object recognition task. In a first dose–response study, L-NAME, at 30 but not at 10 mg/kg impaired the animals' performance, whereas at 60 mg/kg, it induced side-effects. Molsidomine, 4 mg/kg, antagonized the L-NAME-induced performance deficits. These results indicate that NO is involved in post-training memory processes.

Keywords: Nitric oxide (NO); L-NAME (N^{ω}-nitro-L-argininemethylester); Molsidomine; Recognition memory, rat

1. Introduction

Nitric oxide (NO) is a novel retrograde intracellular messenger in the brain (Garthwaite, 1991) which seems to be involved in the mechanisms of synaptic plasticity, including long-term potentiation in the hippocampus and cognition (Prast and Philippu, 2001).

Behavioral investigations have demonstrated that N^{ω}-nitro-L-argininemethylester (L-NAME), an inhibitor of NO synthase (NOS), disrupted animals' performance in learning tasks (Chapman et al., 1992; Bohme et al., 1993; Estall et al., 1993). Conversely, the NO donors, *S*-nitroso-*N*-acetylpenicillamine, sodium nitroprusside and molsidomine, attenuated learning deficits induced by the NOS inhibitors *N*-nitroarginine (NO-Arg) and 7-nitroindazole (Fin et al., 1995; Huang and Lee, 1995; Meyer et al., 1998).

Among NO donors, molsidomine has a high bioavailability, a long-lasting duration of action (Boger et al., 1994), and lacks overt side-effects at doses displaying an antiamnestic action (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 our own (Rigamonti et al., 2001), and other (Maccario et al., 1997), studies showing the unequivocal neuroendocrine and orexigenic effects of this compound when it is administered systemically. In addition, it is also known that molsidomine increases the permeability of the blood-brain barrier (Mayhan, 2000).

The aim of our study was to investigate the effects of L-NAME on recognition memory and the efficacy of molsidomine in counteracting potential L-NAME-induced performance deficits. For this purpose, the object recognition task, a non-rewarded paradigm based on the spontaneous exploratory behavior of rats, which reflects working memory, was selected (Ennaceur and Delacour, 1988).

Moreover, in an attempt to exclude possible adverse effects of repeated injections of L-NAME and molsidomine, the effects of a single injection of each compound on cognition were evaluated.

2. Materials and methods

Procedures involving animals and their care were conducted in conformity with the international guidelines

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in compliance with national and international laws and policies.

2.1. Subjects

Male (3-month-old) CD-COBS rats (Charles River, Calco, Italy), weighing 300–350 g, were used in this study. The animals were housed in Makrolon cages (35 × 45 × 20 cm), 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 were performed by experimenters who were unaware of the pharmacological treatment.

2.2. Drugs

L-NAME (Sigma, St. Louis, MO, USA) and *N*-[ethoxycarbonyl]-3-[4-morpholinosydnomine] (molsidomine) (Sigma Tau, Milan, Italy) were dissolved in saline (NaCl 0.9%) and injected intraperitoneally, in a volume of 0.5 ml per rat. Doses of molsidomine were chosen on the basis of a study in which they exerted antiamnestic action and did not produce adverse side-effects (Meyer et al., 1998). Doses of compounds are expressed as bases. Control animals received isovolumetric amounts (0.5 ml) of the vehicle (NaCl 0.9%).

2.3. Object recognition task

2.3.1. Apparatus

The test apparatus consisted of a dark open box made of plexiglass (80 × 50 × 60 h cm), which was illuminated by a 60-W lamp suspended 60 cm above the box. The objects to be discriminated 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, they 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 the apparatus and was left to explore these two identical objects. After T1, the rat was put back in its cage and an intertrial interval (ITI) of 60 min was given. Subsequently, the "choice" trial (T2), was performed. During T2, a new object (*N*) replaced one of the samples presented in T1, then, the rats were 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. 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 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 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).

2.3.3. Effects of L-NAME on object recognition memory

Rats were randomly divided into four experimental groups (eight rats per group) as follows: vehicle; L-NAME 10 mg/kg; L-NAME 30 mg/kg; and L-NAME 60 mg/kg. The drugs or the vehicle were given intraperitoneally, immediately after T1.

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

Rats 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 L-NAME 30 mg/kg; L-NAME 30 mg/kg plus molsidomine 2 mg/kg; and L-NAME 30 mg/kg plus molsidomine 4 mg/kg. The drugs or the vehicle were given intraperitoneally, immediately after T1.

2.4. Statistical analysis

Data are expressed as mean ± S.E.M. Preference of animals for objects or locations was analyzed by the

Student's *t*-test for each experimental group. Results of experiment 1 (exploration times and discrimination index *D*) were evaluated by the one-way analysis of variance (ANOVA) followed by the Tukey's post-hoc test. Data of experiment 2 were assessed by the two-way ANOVA followed by the Tukey's post-hoc test.

3. Results

No difference within any group when the exploration time was compared according to the nature of objects and their locations in the apparatus was observed.

3.1. Experiment 1: effects of L-NAME on object recognition memory

L-NAME given at 60 mg/kg reduced motility in the rats, hence, this dose was discarded, and the test was performed with doses of 10 and 30 mg/kg.

Analysis of exploration levels during T2 did not reveal any difference among the various groups. Discrimination index *D* results showed that the vehicle and the 10 mg/kg L-NAME-treated rats discriminated better *N* than *F* during T2 with respect to their counterparts which received 30 mg/kg L-NAME; [$F(2,21)=11.9$, $P<0.01$; Tukey's test, $P<0.05$ vs. L-NAME 30 mg/kg group, Table 1].

3.2. Experiment 2: effects of molsidomine on L-NAME-induced deficits in the object recognition task

During T2, no differences in total exploration time were seen among the different groups (Fig. 1A). Discrimination index *D* data revealed a significant main effect of L-NAME [$F(5,54)=134.3$, $P<0.01$], of molsidomine [$F(5,54)=14.8$, $P<0.01$], and a significant interaction between L-NAME and molsidomine [$F(5,54)=9.8$, $P<0.01$, Fig. 1B]. Rats, except those treated with vehicle plus L-NAME and L-NAME plus molsidomine 2 mg/kg, discriminated significantly better *N* than *F*; (Tukey's test, $P<0.05$ vs. vehicle plus L-NAME and L-NAME plus molsidomine 2 mg/kg).

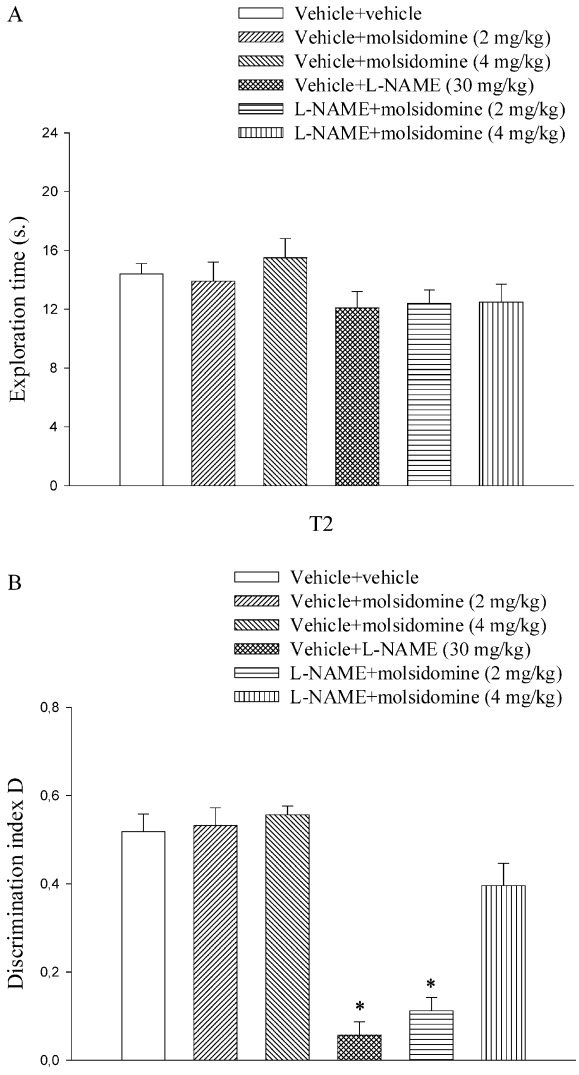

Fig. 1. Results are expressed as mean ± S.E.M. (A) L-NAME and molsidomine were injected intraperitoneally, immediately after T1. Total exploration time displayed by different groups of rats during T2. (B) Discrimination index *D* performance expressed by different groups of rats during T2. * $P<0.05$ vs. all the other groups.

Table 1
Effects of different doses of L-NAME on object recognition in the 3-month-old rat

Group	*N*	T2 total exploration time (s) mean ± S.E.M.	T2 discrimination index (*D*) mean ± S.E.M.
Vehicle	8	13.8 ± 0.9	0.466 ± 0.007
L-NAME (10 mg/kg)	8	12.9 ± 1.4	0.450 ± 0.090
L-NAME (30 mg/kg)	8	12.7 ± 1.7	0.110 ± 0.055[a]

N = number of rats; vehicle and L-NAME were injected intraperitoneally immediately after T1.

[a] $P<0.05$ vs. all the other groups.

4. Discussion

An inhibitory effect of L-NAME on memory was observed at the 30-mg/kg dose. The dose of 10 mg/kg was ineffective, and at 60 mg/kg, induced hypomotility, leading to exclusion of the latter results from the experiments.

Molsidomine, at 4 but not at 2 mg/kg, successfully counteracted the L-NAME-induced-performance deficits. Molsidomine (and L-NAME) influenced rats' performance during retention, seemingly reflecting a modulation of post-training mnemonic processes (storage and/or retrieval of information). However, in our study, treatment was per-

formed just after T1, but a 60-min ITI was given. It is therefore impossible to dissect on which specific memory component (storage or retrieval) either drug was acting.

Despite these limitations, our results agree with previous findings in which the involvement of NO in post-training memory stages has been disclosed using different modalities (Fin et al., 1995; Kopf and Baratti, 1996; Prickaerts et al., 1997; Blokland et al., 1998; Pitsikas et al., 2002).

L-NAME and molsidomine were delivered systemically; therefore, it cannot be excluded that nonspecific factors might have influenced animals' performance. No difference in exploration levels during T2 was observed among the different groups, implying that the effects of L-NAME and molsidomine on rats' cognitive performance were unrelated to the extent of general exploratory behavior.

Another problem when using NOS inhibitors or donors relates to their reciprocal effects on blood pressure, NOS inhibitors being hypertensive, NO donors, hypotensive. It is difficult, therefore, to quantify how and to what extent these cardiovascular effects might have specifically affected cognition. Reportedly, NOS inhibitors injected systemically, induce a nearly maximal hypertensive effect at 10 mg/kg (Rees et al., 1990), a dose which, in the present study, did not affect rats' performance (either exploratory levels or cognitive performance). Therefore, behavioral consequences due to a potential L-NAME-induced hypertensive effect may be excluded. Collectively, our findings are in line with a previous study in which L-NAME up to 20 mg/kg was found ineffective on sensorimotor or motivational measures (Estall et al., 1993). For molsidomine, the doses used in our study were very low and, allegedly, devoid of side-effects (Meyer et al., 1998).

The mechanism of action of NO donors on cognition is still under investigation. The hypothesis that these compounds can enhance memory by improving cerebral blood flow has not been supported by sound results (Patel, 1995).

Finally, that a state dependency underlies the amnestic effects of 7-nitroindazole has been reported (Blokland et al., 1998). However, the effects of L-NAME on cognition would be unrelated to the induction of a state dependency (Kopf and Baratti, 1996; Xu et al., 1999), and in addition, prior results of ours ruled out the possibility of a state-dependent-mediated mechanism also for molsidomine (Pitsikas et al., 2002).

In summary, studies herein presented indicate that L-NAME impairs recognition memory in the rat, an effect which is antagonized by molsidomine, in all suggesting that NO is involved in post-training memory processes.

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