Pharmacology, Biochemistry and Behavior 138 (2015) 111–116

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

The nitric oxide donor molsidomine induces anxiolytic-like behaviour in two different rat models of anxiety

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ARTICLE INFO

Article history:
Received 29 May 2015
Received in revised form 25 August 2015
Accepted 2 September 2015
Available online 9 September 2015

Keywords:
Nitric oxide
Molsidomine
Diazepam
Light/dark test
Open field test
Motility
Anxiety
Rat

ABSTRACT

Experimental evidence indicates the implication of the nitric oxide (NO) in anxiety. Contradictory results were reported however, concerning the effects of NO donors in animal models of anxiety disorders. The present study investigated the effects of the NO donor molsidomine on anxiety-like behaviour and compared them with the anxiolytic diazepam in rats. For this purpose, the light/dark and the open field tests were used. The effects of molsidomine on motility were also assessed. Intraperitoneal (i.p.) administration of molsidomine (1 and 4 mg/kg) did not influence rats' performance either in the light/dark or in the open field test. Administration of 2 mg/kg molsidomine significantly prolonged the time spent in the light chamber in the rats compared with the vehicle-treated animals, did not affect the first latency to enter the dark chamber and did not influence the number of transitions between the light and dark compartments of the apparatus. In the open field test, rats that received 2 mg/kg molsidomine spent more time in the central zone of the apparatus and exhibited an increment of rearing episodes compared with control and to molsidomine 1 and 4 mg/kg-treated rats. Nevertheless, molsidomine, at any dose tested, did not alter locomotor activity compared with vehicle-treated rats in a motility test. The present results indicate that the 2 mg/kg molsidomine induced anxiolytic-like effects in the light/dark and open field tests in the rat cannot be attributed to changes in locomotor activity. The magnitude of the molsidomine (2 mg/kg)-induced anxiolytic-like effects was not different to that produced by the benzodiazepine anxiolytic diazepam (1 mg/kg).

1. Introduction

Nitric oxide (NO), a small, short-lived, and highly diffusible gas, is an important intra- and inter-cellular messenger in the brain. Several physiological functions have been attributed to NO and it has also been implicated in various pathological states (Garthwaite, 1991; Dawson and Snyder, 1994). Activation of N-methyl-D-aspartate (NMDA) receptors has been shown to induce NO synthesis. NO reportedly is involved in the mechanisms of synaptic plasticity (O'Dell et al., 1991) and plays an important role in cognition (Prast and Philippu, 2001).

The implication of NO in anxiety has been proposed although its role in this pathology has not yet been fully clarified (Li and Quock, 2002; Guimaraes et al., 2005). Little and contradictory information is available concerning the effects of NO donors on anxiety. Specifically, anxiolytic effects following administration of NO donors were evidenced in studies performed in mice (Li and Quock, 2002; Masood et al., 2009). In contrast, anxiogenic or no effects were revealed after intrahippocampal administration of NO donors in studies carried out in rats (Ferreira et al., 1999; Calixto et al., 2010). Thus, additional research is required to clarify the effects of NO donors on anxiety-like behaviour.

Among NO donors, molsidomine has a high bioavailability, a long-lasting duration of action, likely crosses the blood–brain barrier (Boger et al., 1994) and increases its permeability (Mayhan, 2000). Molsidomine was found to display antiamnestic action in the rat (Pitsikas et al., 2006; Pitsikas and Sakellaridis, 2007) and to potentiate the anticonvulsant action of different NMDA antagonists, of riluzole and valproate in the mouse (Tutka et al., 2002a, 2002b). At the moment, no studies have addressed the effects of molsidomine on anxiety-like-behaviour in rats.

Considering the aforementioned evidences, the aim of the present study was to investigate the effects of molsidomine on anxiety-like-behaviour in the rat using different experimental approaches; the light/dark box and the open field tests. The light/dark box test is a procedure that is based on the innate aversion of rodents to brightly illuminated areas and the conflicting tendency of rodents to explore novel environments (Crawley and Goodwin, 1980). The open field test involves encounter with a novel environment and gives rise to behavioural and physiological reactions related to anxiety (Prut and Belzung, 2003). Further, locomotor activity was also assessed as an independent measure of the potential motoric effects of the compound that could influence rats' performance in the light/dark box and the open field tests

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http://dx.doi.org/10.1016/j.pbb.2015.09.004
0091-3057/

(Crawley, 1985). Finally, the potential anxiolytic effects of molsidomine were compared with those displayed by the positive control diazepam in the light/dark box test.

2. Materials and methods

2.1. Animals

Male 3-month-old albino Wistar rats (Hellenic Pasteur Institute, Athens, Greece) that weighed 250–300 g were used in this study. The animals were housed in Makrolon cages (47.5 cm length × 20.5 cm height × 27 cm width) three per cage, in a climate-regulated environment (21 ± 1 °C; 50–55% relative humidity) under a 12 h/12 h (lights on at 7:00 AM) light/dark cycle with free access to food and water.

The procedures that involved animals and their care were conducted in accordance with international guidelines and 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. 85-23, 1985).

2.2. Drugs

All solutions were freshly prepared on the day of testing and were administered in a volume of 1 ml/kg. Molsidomine (Sigma Tau, Milan, Italy) was dissolved in saline (NaCl 0.9%). Diazepam (Sigma, St. Louis, MO, USA) was suspended in saline containing 0.1% Tween 80. Doses and time of administration of molsidomine were chosen on the basis of previous reports in which they were effective against learning impairments and did not produce adverse side effects (Pitsikas et al., 2006; Pitsikas and Sakellaridis, 2007). Dose and time of administration of diazepam were selected based on a previous study which was found effective in the light/dark test and did not affect rats' locomotor activity (Saito et al., 2013). Control animals received isovolumetric amounts of the vehicle solution (saline) in experiments 1, 2 and 3 while in experiments 4 and 5 they received isovolumetric amounts of saline containing 0.1% Tween 80.

2.2.1. Light/dark test

The light/dark box apparatus consisted of a wooden box (48 cm length × 24 cm height × 27 cm width) divided into two equal-size compartments by a barrier that contained a doorway (10 cm height × 10 cm width). One of the compartments was painted black and was covered with a lid and the other compartment was painted white and illuminated with a 60 W light bulb positioned 40 cm above the upper edge of the box. The test was performed as described previously (Pitsikas et al., 2008). On the test day, the rats were transported to the dimly illuminated (20 lx) test room and remained undisturbed in their home cages for 2 h. Then the animals were placed in the middle of the lit compartment, facing away from the dark chamber. The rats were allowed to freely explore the apparatus for 5 min. The latency to enter (with all four paws) the dark compartment, number of transitions and time spent in the light and dark compartments were recorded. A between-subjects design was used for the factor dose: thus, each rat was tested only once.

2.2.2. Open field test

The test apparatus consisted of a dark open box made of Plexiglas (70 cm length × 50 cm height × 70 cm width). The open field arena was divided-by black lines-into 16 squares of 17.5 × 17.5 cm^2. The central four squares were defined as the central zone, in which animals' activity was regarded as a measure of anxiety (Prut and Belzung, 2003). The test was performed as described previously (Grivas et al., 2013). On the test day, the rats were transported to the dimly illuminated (20 lx) test room and remained undisturbed in their home cages for 2 h. Each animal was then placed in the same corner of the open field arena and its behaviour was recorded for 5 min. The variables observed were: (a) the first latency to enter the central zone of the open field arena, (b) the number of entries in the central zone of the open field arena, (c) the amount of the time spent in the central zone as defined by all forepaws being in the central four squares of the apparatus, (d) the number of squares crossed (i.e., horizontal activity), (e) the number of rearing behaviours (i.e., vertical activity, defined as raising both forepaws above the floor while balancing on hind limbs), and (f) the duration of grooming events. A between-subjects design was used for the factor dose: thus, each rat was tested only once.

2.2.3. Locomotor activity test

Spontaneous locomotor activity was assessed in an activity cage (Ugo Basile, Varese, Italy). The apparatus consisted of a box made of Plexiglas (41 cm length × 33 cm height × 41 cm width). Every movement of the animal produced a signal caused by variations in the inductance and capacitance of resonance circuitry of the apparatus. The signals were then automatically converted into numbers that reflected horizontal activity counts. Changes in activity counts represent a standard behavioural assay for testing the motoric effects of drugs. The test was performed as described previously (Grivas et al., 2013). On the test day, the naive rats were transported to the dimly illuminated (20 lx) test room and remained undisturbed in their home cages for 2 h. Thereafter, each animal was placed into the locomotor activity arena and spontaneous locomotion was recorded for 5 min. Separate cohorts of naive rats were used for the locomotor activity experiment and the light/dark box experiment. A between-subjects design was used for the factor dose. Thus, each rat was tested only once.

2.3. Experimental protocol

Experiments were conducted between 9:00 AM and 3:30 PM during the light phase of the light/dark cycle. To avoid the presence of olfactory cues, all the apparatuses (light/dark box, open field arena and motor activity cage) were thoroughly cleaned with 20% ethanol and then wiped with dry paper after each trial.

Behaviour in the light/dark and open field tests was video-recorded. Data evaluation was subsequently performed by an observer who was unaware of the pharmacological treatment of each subject.

2.3.1. Experiment 1: effects of molsidomine on rats' performance in the light/dark test

Naive rats were randomly divided into four experimental groups (10 rats per group): vehicle, and 1, 2 and 4 mg/kg molsidomine [intraperitoneally (i.p.) 60 min before testing].

2.3.2. Experiment 2: effects of molsidomine on rats' performance in the open field test

Naive rats were randomly divided into four experimental groups (8 rats per group): vehicle, and 1, 2 and 4 mg/kg molsidomine (i.p., 60 min before testing).

2.3.3. Experiment 3: effects of molsidomine on rats' performance in the locomotor activity test

Naive rats were randomly divided into four experimental groups (8 rats per group): vehicle, and 1, 2 and 4 mg/kg molsidomine (i.p., 60 min before testing).

2.3.4. Experiment 4: comparison of the effects of molsidomine with those of diazepam on rats' performance in the light/dark test

In this study, the effective dose of molsidomine (2 mg/kg) in the light/dark test was compared with the anxiolytic compound diazepam in the same behavioural paradigm. Naive rats were randomly divided into three experimental groups (8 rats per group): vehicle, 2 mg/kg molsidomine (i.p., 60 min before testing) and 1 mg/kg diazepam [subcutaneously (s.c.) 30 min before testing].