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Measure of anxiety-related behaviors and hippocampal BDNF levels associated to the amnesic effect induced by MK-801 evaluated in the modified elevated plus-maze in rats

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HIGHLIGHTS

- In the present work we investigated the amnesic effect of MK-801 in the mEPM.
- A behavioral and neurochemical approach was performed.
- Anxiety-related behaviors were not related with the amnesic effect.
- A decrease in hippocampal BDNF levels was found in MK-801-treated animals.
- We provide valuable information related to the amnesic effect of MK-801 in the mEPM.

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ABSTRACT

Non-competitive N-methyl-D-aspartate receptor (NMDA-R) antagonists impair rodent cognition. Specifically, MK-801, the most potent NMDA-R antagonist, induces an amnesic effect on the modified elevated plus maze (mEPM) learning test in rodents, which reflects spatial long-term memory. However, alterations in anxiety-related behaviors could overlap this amnesic effect. Accumulated evidence supports the role of brain-derived neurotrophic factor (BDNF) in learning and memory processes and deficits in hippocampal BDNF function, which underlie cognitive impairments, have been extensively reported. Therefore, we investigated if changes in anxiety-related behaviors and hippocampal BDNF levels are related with the amnesic effect induced by MK-801 in the mEPM.

Transfer latency (TL) as an index of spatial memory in the mEPM was used. TL1 was evaluated 30 min after saline/MK-801 injection (day 1, acquisition session) while learning/memory performance was measured 24 h later at TL2 (day 2, retention session). Also at TL2, two other experimental groups were added to measure the anxiety-related behaviors using the classic EPM and BDNF protein levels by ELISA. To evaluate if amnesia endures, an additional session was recorded on day 3 (TL3) and BDNF levels were measured.

While TL1 was not significantly modified by MK-801, TL2 was increased compared to the control group indicating an amnesic effect. This effect was not mimicked by anxiety-related behaviors and it was associated to a significant attenuation of BDNF levels. During the third post-training day, the cognitive performance of MK-801-treated animals was improved and an increased BDNF protein expression in the hippocampus accompanied this change.

1. Introduction

It has been postulated that non-competitive N-methyl-D-aspartate receptor (NMDA-R) antagonists such as ketamine and phencyclidine (PCP) elicite or exacerbate schizophrenia-like symptoms in healthy subjects or in schizophrenic patients respectively, including positive and negative symptoms as well as cognitive impairments [1–4]. Although it is not consumed by humans, the administration in rodents of the most potent NMDA-R antagonist, dizocilpine or MK-801 induces behavioral alterations which resemble those described above for NMDA antagonists [1,2,5]. Cognitive dysfunction is being increasingly studied in schizophrenia since it is considered the most prevalent symptom as well as it can significantly decrease the patient's quality of life [6]. Therefore, it is now accepted that NMDA antagonists are widely used as animal models of cognitive impairments associated to schizophrenia (CIAS) [7,8]. Several evidences have shown memory-impairing effects

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induced by MK-801 using the modified elevated plus-maze (mEPM) learning test, a non-sophisticated and straightforward paradigm commonly used in rodents [9–15]. Indeed, some of these studies also investigated the antipsychotic action on the impairment of memory function induced by MK-801 [10,15]. As the same paradigm allows the detection of anxiolytic or anxiogenic-like behavioral responses, false positive/negative effects on learning/memory function could be produced and anxiety-related behaviors might mimic or interfere with a cognitive impairment. Besides, it has been reported that systemic or intrahippocampal injections of MK-801 in rats can induce anxiolytic effects using the EPM [16–18].

The BDNF (brain-derived neurotrophic factor) is a member of the neurotrophin family and has been commonly related to cognitive functions, especially learning and memory processes [19–23]. Strong evidence in humans and knock-out mice suggest that BDNF plays a role in the hippocampal function and hippocampal-dependent memory [22–25]. It has been reported that hippocampal-specific deletion of the BDNF gene impairs novel object recognition as well as spatial learning in the water maze [26]. Moreover, a relationship between cognitive impairments induced by MK-801 and alterations in BDNF mRNA or protein levels in rat hippocampus has been studied [27–29]. However, as far as we are concerned, no relation has been provided between hippocampal BDNF level and the amnesic effect induced by M-801 evaluated in the mEPM learning test. Additionally, no information about the duration of this cognitive impairment has been provided using this paradigm.

The present paper was designed to investigate whether anxiety-related behaviors and BDNF levels in the hippocampus are related to the learning/memory deficit induced by MK-801 in rats evaluated in the mEPM learning test. The duration of the effect was also analyzed.

2. Material and methods

2.1. Animals

Fifty two adult male Wistar rats weighing 260–320 g, bred in the IIBCE animal facilities (Montevideo) were employed in the study. The animals were housed in groups of 6 in plastic cages (50 × 37.5 × 21 cm) with food and water available ad libitum and kept under controlled conditions (temperature 22 ± 2 °C, 12-h light–dark cycle, lights on at 7:00 A.M.). All procedures were carried out in accordance to the IIBCE Bioethics Committee's requirements and under the current ethical regulations of the national law on animal experimentation N°18.611. Adequate measures were taken to minimize animals' discomfort or stress, and all efforts were made in order to use the minimal number of animals necessary to produce reliable scientific data.

2.2. Drug and dosage

(+)-MK-801 [dizocilpine (5R,10S)-(1)-5-methyl-10,11-dihydro-5H-dibenzo [a,d] cyclohepten-5,10-imine hydrogen maleate)] was obtained from Sigma RBI. In all the experiments, MK-801 was administered intraperitoneally (i.p.) at 0.05 mg/kg, a dose below the threshold to produce the typical behavioral motor syndrome of NMDA-R antagonists [30,31]. This low dose was chosen on the basis of previous studies showing an amnesic effect of MK-801 at 0.15 mg/kg [11–13].

2.3. Behavioral measures

Rats were taken to the experimental room in their home cages, identified and weighed one day before the behavioral experiments to allow acclimation to the test environment. The experimental room was under controlled temperature (22 ± 2 °C) and the behavioral testing was conducted using the mEPM learning test [9,15], the classical elevated plus-maze paradigm [32,33] and the open field test, as previously described [34,35]. All injections were done at a volume of 1 ml/kg and all experiments were performed between 8:00 a.m. and 1:00 pm.

2.3.1. mEPM learning test

Learning/memory function assessment was done using the mEPM learning test, which measures spatial long-term memory [9,15]. Transfer latency (TL, the time at which animals move from the open arm to the closed arm) was used as an index of learning and memory processes. The elevated plus-maze was made of wood and consisted of 2 open arms (50 × 10 cm) surrounded by a short (1 cm) wooden edge to avoid falls, and 2 closed arms (50 × 10 × 40 cm) arranged so that the 2 open arms were opposite each other. The arms were connected by a central platform (10 × 10 cm). The maze was elevated to a height of 50 cm above the floor. To avoid confounding olfactory cues, the maze was cleaned with an alcohol–water solution (30%) after each rat. The mEPM procedure was performed accordingly to previous studies [11–13] with some modifications. Animals were randomly assigned to the different experimental and control groups. Briefly, 30 min after saline or MK-801 (0.05 mg/kg) i.p. injection, rats were placed in the open arm and the time to reach the closed arms was counted in day 1 (TL1, acquisition session) and day 2 without drug injection (TL2, retention session). After entering the closed arm, the rat was allowed to freely move in the maze for 10 s and then returned to the home cage. Rats not entering the closed arm within 90 s were excluded from further experiments. Training (repeated exposure of an animal to the open arms) shortens TL variable as a consequence of learning and memory acquisition and retention. Contrastingly, increments in TL2 variable indicate impairments in animal learning/memory function. In order to evaluate if the NMDA-R antagonist induced a long-lasting effect on transfer latency values, an additional session was recorded on day 3 (TL3). This measure was originally included in the present study since it was not provided by Hlinák and collaborators.

2.3.2. Classical EPM anxiety test (cEPM)

According to previous studies, the administration of MK-801 is expected to induce an amnesic effect which is indicated by an increase in TL2 with respect to control group evaluated in the mEPM learning test. However, this response could be mimicked or confused by a putative anxiolytic response represented by an increase in the time spent on the open arm (see Introduction). In order to discard this hypothesis, an additional study was carried out. Another group of animals was i.p. injected with the NMDA-R antagonist (0.05 mg/kg) or saline on day 1 and 24 h later and the effects on experimental anxiety were evaluated 24 h later, using the same elevated plus-maze apparatus described above. This evaluation time was in accordance with that used to evaluate TL2 in the mEPM learning test. Open and closed entries and time spent in both arms were recorded and expressed as percentage [32,33,36].

2.3.3. Open field test (OFT)

As MK-801 may alter animal locomotion giving false-positive/negative effects in the mEPM learning test, a monitoring of motor activity was carried out during the first day, 5 min before starting the mEPM learning test. Animal motor activity was automatically assessed in an open field chamber without habituation and beginning 25 min after saline or MK-801 (0.05 mg/kg) i.p. injection. The horizontal motor activity, defined as the total distance traveled in meters (m), was recorded during 5 min using a digital camera associated to a video-tracking Ethovision software 7.0 (Noldus, The Netherlands).

2.4. BDNF measurement

Two different groups of animals were used in order to establish an association between the BDNF protein levels and animal behavior recorded during TL2 and TL3. One group of rats was i.p. injected with the MK-801 (0.05 mg/kg) or saline on day 1. Thirty minutes later,