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Clozapine blockade of MK-801-induced learning/memory impairment in the mEPM: Role of 5-HT$_{1A}$ receptors and hippocampal BDNF levels

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ABSTRACT

Cognitive impairment associated with schizophrenia (CIAS) is highly prevalent and affects the overall functioning of patients. Clozapine (Clz), an atypical antipsychotic drug, significantly improves CIAS although the underlying mechanisms remain under study. The role of the 5-HT$_{1A}$ receptor (5-HT$_{1A}$-R) in the ability of Clz to prevent the learning/memory impairment induced by MK-801 was investigated using the modified elevated plus-maze (mEPM) considering the Transfer latency (TL) as an index of spatial memory. We also investigated if changes in hippocampal brain-derived neurotrophic factor (BDNF) levels underlie the behavioral prevention induced by Clz.

Clz (0.5 and 1 mg/kg)- or vehicle-pretreated Wistar rats were injected with MK-801 (0.05 mg/kg) or saline. TL was evaluated 35 min later (TL1, acquisition session) while learning/memory performance was measured 24 h (TL2, retention session) and 48 h later (TL3, long-lasting effect). WAY-100635, a 5-HT$_{1A}$-R antagonist, was pre-injected (0.3 mg/kg) to examine the presumed 5-HT$_{1A}$-R involvement in Clz action. At TL2, another experimental group treated with Clz and MK-801 and its respective control groups were added to measure BDNF protein levels by ELISA.

TL1 and TL3 were not significantly modified by the different treatments. MK-801 increased TL2 compared to control group leading a disruption of spatial memory processing which was markedly attenuated by Clz. WAY-100635 suppressed this action supporting a relevant role of 5-HT$_{1A}$-R in the Clz mechanism of action to improve spatial memory dysfunction. Although a significant decrease of hippocampal BDNF levels underlies the learning/memory impairment induced by MK-801, this effect was not significantly prevented by Clz.

1. Introduction

Positive symptoms are considered the primary target in the treatment of schizophrenia. However, schizophrenia is also associated with negative symptoms and cognitive dysfunction, which are very poorly treated by antipsychotic drugs [1,2]. Attention, working memory and long-term memory dysfunctions are the most common cognitive impairments associated with schizophrenia (CIAS) [3]. CIAS can significantly decrease the patient's life quality, so effective treatments may substantially impact the patient's general functioning [4].

Although it is frequently assumed that atypical antipsychotics therapy (e.g. clozapine, ziprasidone, quetiapine and olanzapine) is superior over to classical antipsychotics (e.g. haloperidol) for enhancing cognition [5–8], the neural mechanisms involved have not been fully elucidated yet. Moreover, some clinical studies have not shown significant differences between first- and second-generation psychotropic drugs in the improvement of cognitive deficits [9,10]. Varying effects on normal cognitive functions have been observed when atypical antipsychotics were used in preclinical cognitive tests. In some cases, antipsychotics disturbed cognitive functions, whereas these agents produced no effect in other studies [11–13]. The necessity of new drugs and pharmacological strategies for treating CIAS is still a major goal.

Several serotonin (5-HT) receptors are targeted by atypical antipsychotic drugs [14–16]. Among the various 5-HT receptors involved in cognitive functions, 5-HT$_{1A}$ receptors (5-HT$_{1A}$-Rs) are of special interest because they are abundant in brain areas associated with cognitive functions, such as the cerebral cortex, hippocampus and septum [17–19]. Indeed, there are several pieces of evidence proposing these molecular sites therapeutically relevant to improve CIAS [20–23].

Clozapine (Clz), the gold standard among atypical antipsychotics, interacts with multiple receptors showing higher affinity for serotonergic 5-HT$_{2A}$ than for dopaminergic D2 receptors. Additionally, Clz acts as a weak partial agonist at 5-HT$_{1A}$-Rs [14,16,24]. Even modest 5-HT$_{1A}$-Rs activation, it was demonstrated that Clz increases prefrontal

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cortex (PFC) dopamine (DA) release, via 5-HT_{1A}-Rs but not 5-HT_{2A}-Rs [25–29]. Indeed, this neurochemical capacity has been attributed to its superior clinical efficacy in the treatment of negative and cognitive symptoms [26,28,30–33]. Interestingly, it was demonstrated that 5-HT_{1A}-Rs, but not 5-HT_{2A}-Rs, are required for the reversal of phencyclidine (non-competitive NMDA-R antagonist) effect by Clz on slow cortical oscillations in PFC [34]. Moreover, Clz was able to inhibit the motor hyperactivity induced by MK-801 equally in wild-type and 5-HT_{1A}-R knock-out mice, indicating that Clz ability to block this behavioral effect (thought to underlie positive symptoms) did not depend on 5-HT_{1A}-R activation [35].

Non-competitive NMDA receptors (NMDA-Rs) antagonists are used as pharmacological models of schizophrenia due to their ability to evoke schizophrenia symptoms as well as cognitive dysfunction [36–38]. NMDA-Rs antagonists also disturb learning and memory functions in animals. Actually, they are widely used as animal models of CIAS [39,40]. In a previous study we demonstrated that the acute administration of MK-801, the most potent non-competitive NMDA-Rs antagonist, induced a learning/memory impairment evaluated in the modified elevated plus maze (mEPM), an effect that was directly associated to a low level of brain-derived neurotrophic factor (BDNF) in the hippocampus [41]. Therefore, we proposed the predictive validity of MK-801-induced learning/memory impairment in the mEPM as a preclinical model to study CIAS and putative treatments [41]. Indeed, some studies have already investigated the antipsychotic action on the impairment of cognitive function induced by MK-801 using the mEPM [42–44]. However, the effect of Clz on MK-801-induced learning/memory impairment and the study of its mechanism of action was not investigated so far using the mEPM paradigm.

The present paper was designed to investigate whether 5-HT_{1A}-Rs participate in the ability of Clz to antagonize a learning/memory deficit induced by MK-801 in rats using the mEPM paradigm. Given that the alteration in BDNF expression has been related to learning and memory processes [45–47] and schizophrenia [48], the role of this molecular parameter was also studied underlying the Clz action as a common mechanism to improve cognitive function.

2. Methods and materials

2.1. Animals

One hundred and 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 (followed the National Institutes of Health guide for the care and use of Laboratory animals; NIH Publications No. 8023, revised 1978) and under the current ethical regulations of the national law on animal experimentation N°18.611. Adequate measures were taken to minimize discomfort or stress of the animals, and all efforts were made to use the minimal number of animals necessary to produce reliable scientific data.

2.2. Drugs

(+)-MK-801 [dizocilpine (5*R*,10*S*)-(1)-5-methyl-10,11-dihydro-5H-dibenzo [a,d] cyclohepten-5,10-imine hydrogen maleate] and WAY-100635 were obtained from Sigma RBI and Clozapine from Tocris. MK-801 and WAY-100635 were dissolved in saline; clozapine was dissolved in a minimal volume of HCl 0.1 M, and diluted with saline (pH-adjusted to 5–6 with NaOH). Aliquots were prepared and stored at − 20 °C. Clozapine vehicle was also stored at − 20 °C. Drugs were injected intraperitoneally (i.p). or subcutaneously (s.c.) and control groups received the corresponding saline or vehicle injection.

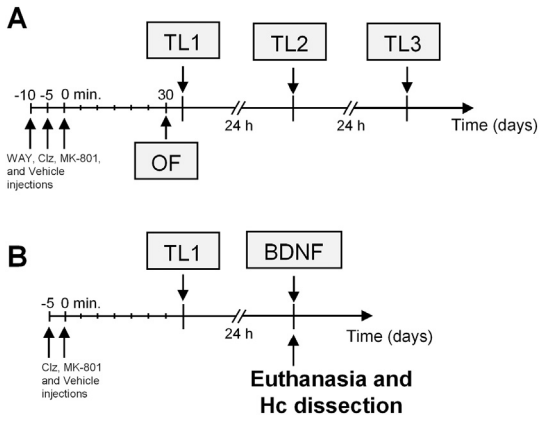

Fig. 1. Experimental protocols: In A learning/memory function was assessed in the mEPM paradigm considering the transfer latency (TL) as an index of spatial memory. Drugs or corresponding vehicles were administrated 40, 35 and 30 min prior to locomotor activity assessment (5 min) in the Open Field (OF). Immediately acquisition session was performed, where TL1 was recorded. Twenty four and 48 h later TL2 and TL3 respectively, were registered. In B an independent group of rats was used to measure BDNF protein levels associated to Clz prevention of the amnesic effect induced by MK-801 evaluated in mEPM. Drugs or corresponding vehicles were administrated 40 and 35 min prior TL1 recording. Twenty four h later animals were euthanatized for hippocampus (Hc) dissection.

2.3. Experimental procedures

Rats were brought in their home cages to the experimental room, 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 [41,49] and the open-field (OF) test, as previously described [41,50,51].

2.3.1. mEPM learning test

Learning/memory function assessment was done using the mEPM learning test, which measures spatial long-term memory [41,49]. 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 (Fig. 1A). The mEPM procedure was performed accordingly to previous studies with modifications [41]. Animals were randomly assigned to the different experimental and control groups. Briefly, rats were placed in the open arm and the time to reach the closed arms was counted in day 1 (TL1, acquisition session), day 2 (TL2, retention session) and day 3 (TL3, long-lasting effect) [41]. 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 TL2 variable as a consequence of learning acquisition and retention or consolidation of memory [44,52].

2.3.2. Open field test

Since the compounds assayed may alter animal locomotion or experimental anxiety and give false-positive/negative effects in the mEPM learning test, a monitoring of locomotor activity and the time spent in center and periphery zones of an open field (OF) chamber was carried out during the first day, 5 min before starting the mEPM learning test. Animal activity was automatically assessed in the OF without habituation and beginning 30 min after saline or the last drug injection in each experimental group (see the following item; Fig. 1A). The horizontal locomotor activity, defined as the total distance travelled in meters (m), and the time spent in center and periphery zones (in seconds) were automatically recorded by a camera connected to a