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Effects of dizocilpine-induced glutamatergic blockade in the nucleus accumbens septi on the plus maze test

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Abstract

Background: In previous studies, we have observed that specific *N*-methyl-D-aspartic acid (NMDA) antagonists and non-NMDA antagonists injected within the nucleus accumbens septi (NAS) induced an anxiolytic-like effect in the plus maze test in rats. In the present study, the effect of intracanalicular blockade of NMDA receptors using dizocilpine in the plus maze was studied in male rats bilaterally cannulated NAS.

Methods: Rats were divided into five groups that received either 1 µL injections of saline or dizocilpine (MK-801, [5R,10S]-[+]-5-methyl-10,11-dihydro-5H-dibenzo [*a*,*d*] cyclohepten-5,10-imine) in different doses (0.5, 1, 2, or 4 µg) 15 min before testing.

Results: Time spent in the open arm increased under dizocilpine treatment with the two higher doses (2 and 4 µg, $p < 0.05$), extreme arrivals were increased by the three higher doses (1 µg, $p < 0.05$; 2 and 4 µg, $p < 0.01$), and open arm entries by the three higher doses (1, 2, and 4 µg, $p < 0.05$). A dose-effect relationship was observed in all cases.

Conclusions: We conclude that dizocilpine-glutamatergic blockade in the accumbens lead to an anxiolytic-like effect and a behavioral disinhibition related to an increase in some motoric parameters, showing specific behavioral patterns.

Keywords: accumbens; anxiety; dizocilpine; plus maze; rat.

Introduction

Nucleus accumbens septi (NAS) of the basal forebrain is considered the main component of the ventral striatum of the rat [1]. It has attracted interest due to its postulated role in depressive disorders [2], schizophrenia and antipsychotic drugs mechanisms [3–5], and stereotyped behavior [6]. It integrates afferences from different areas. It is the case of dopaminergic projections originating from the ventral tegmental area, and is related to reward [7]. Glutamatergic projections reach this nucleus coming from the amygdala, hippocampus, and prefrontal cortex [8]. We have recently suggested that NAS could be considered a brain region involved in anxiety integration, and is related to affective flattening in schizophrenia [3, 4, 9–12]. Additionally, some efferences from NAS have been described to have an inhibitory effect on dorsomedial thalamic nuclei afferences, necessary to process relevant stimuli [3, 4, 8], suggesting that NAS plays a role in cognitive processes [9, 13–15].

Glutamatergic *N*-methyl-D-aspartic acid (NMDA) receptor antagonists have been shown to have anxiolytic-like effects when administered systemically in a wide range of rodent models [9, 10]. When intra-accumbens injections were made using NMDA receptor blockers, an anxiolitic-like effect was also observed in rats in three different models of anxiety: the open field test, the Vogel test [16], and the plus maze test [9]. Dizocilpine (MK-801,

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[5R,10S]-[+]-5-methyl-10,11-dihydro-5H-dibenzo [*a*,*d*] cyclohepten-5,10-imine) is a drug classically linked to the phencyclidine/sigma receptor blocking the NMDA receptor intracanalicularly [17] such as ketamine and phencyclidine [18, 19]. The aim of the present report is to study the acute effects of dizocilpine on the glutamate phencyclidine/sigma receptor site injected into the NAS in the plus maze anxiety test in rats.

Materials and methods

Subjects

The animals used were male rats from a Holtzman-derived colony, aged 90 days and weighing 240–270 g (n = 105). These rats were maintained under controlled temperature (22 °C–24 °C) and lighting (05:00–19:00 h) conditions. Following the criteria of our laboratory, standard rat chow and water were freely available.

Surgery

Surgery was performed maintaining the animals under ether anesthesia and rats were stereotaxically implanted with bilateral stainless steel cannulae into the NAS. Coordinates used for cannulae implantation were: anterocaudal: +3.4; lateral: ±2.0; vertical: −4.5 [20]. The cannulae consisted of an outer guiding cannula stainless steel tubing (23-gauge, 15 mm in length) provided with an inner removable stylet (30-gauge, 15 mm in length), aiming to prevent obstruction. As in previous studies in our laboratory, rats were housed individually after surgery and maintained undisturbed for a week-long recovery.

Apparatus

The plus maze used in our laboratory is made of wood and consists of two open arms, 50 × 10 cm (length × width), and two enclosed arms 50 × 10 × 50 cm (length × width × height), arranged such that the arms of each kind are opposite each other. The maze is elevated 50 cm above the floor level. In all cases, the room is illuminated by a 60 W bulb, 1.5 m above the apparatus.

Procedure

Animals were manually restrained and injected 15 min before testing. A 30-gauge, 17-mm-long stainless steel injection cannula (dimensioned to reach precisely the NAS) attached to a 10 μL microsyringe (Hamilton Syringes, Merck, Darmstadt, Germany) was introduced into the guide cannula, and 1 μL volume solutions were gradually injected over 2-min periods into both the left and right NAS. The injection cannulae were left in place for an additional period of 1 min to allow for diffusion. As mentioned previously, rats received bilateral injections of saline or drugs (see Histology) 15 min before each session.

Once the experiment was started, rats were placed individually in the center of the plus maze apparatus, facing the open arm, and allowed 5 min for free exploration. Sessions were all carried between 17:00 and 19:00 h, and each rat was used only one time. We recorded some standard measures and some ethological variables [6, 21, 22]. Standard measures included time spent in the open arm, open arm entries, time per entry (quotient between the time spent in the open arm and the number of entries into open arms), closed arm entries (all four paws in a given arm), and quotient between open and closed arm entries [11, 12, 23].

The ethological items recorded were face grooming, head grooming, rearing, end arrivals, and defecation (considered counting the number of fecal boli expelled). Face grooming was defined as washing with forepaws from the snout to the ears. Head grooming was evaluated as counting washes from the ears to the neck. Rearing was considered as a partial or complete standing on the hind limbs. The number of events was recorded to measure grooming and rearing. The extreme arrivals were defined as the number of times the rat reached the end of an open arm.

Drugs

Rats were divided into five groups: saline control (1 μL, n = 25) and dizocilpine (MK-801, Tocris, Bristol, UK) at doses of 0.5 (n = 14), 1.0 (n = 20), 2.0 (n = 24), and 4 μg/1 μL (n = 22), dissolved in saline.

Histology

When the testing was completed, the rats were injected in the NAS with saturated methylene blue solution (1 μL). Fifteen minutes later, they were sacrificed with an excess of ether. The brains were removed from the skull and fixed in 20% formalin solution. Later, the block face was cut and examined with a 10× magnifying lens and the sections containing the injection sites were saved. Microscopic inspection of these sections served to ascertain the location of the cannula tips. The locations were transferred to standard sections taken from a brain atlas [20]. We only report data for those rats that had correct NAS cannula placements (Figure 1).

Data analysis

The Kolmogorov-Smirnov test was used to ascertain normal distribution of data. One-way ANOVA followed by Student-Newman-Keuls test was applied to normally distributed data. In all cases, a $p < 0.05$ (two-tailed) was considered significant. The results are reported as means ± standard errors (n = 14–25).

Bioethical considerations

The animal's care was done according to bioethical and legal dispositions. The housing and experimental procedures were carried out following project approval criteria of the National University of Cuyo, and accordingly to the guidelines set by the European Community

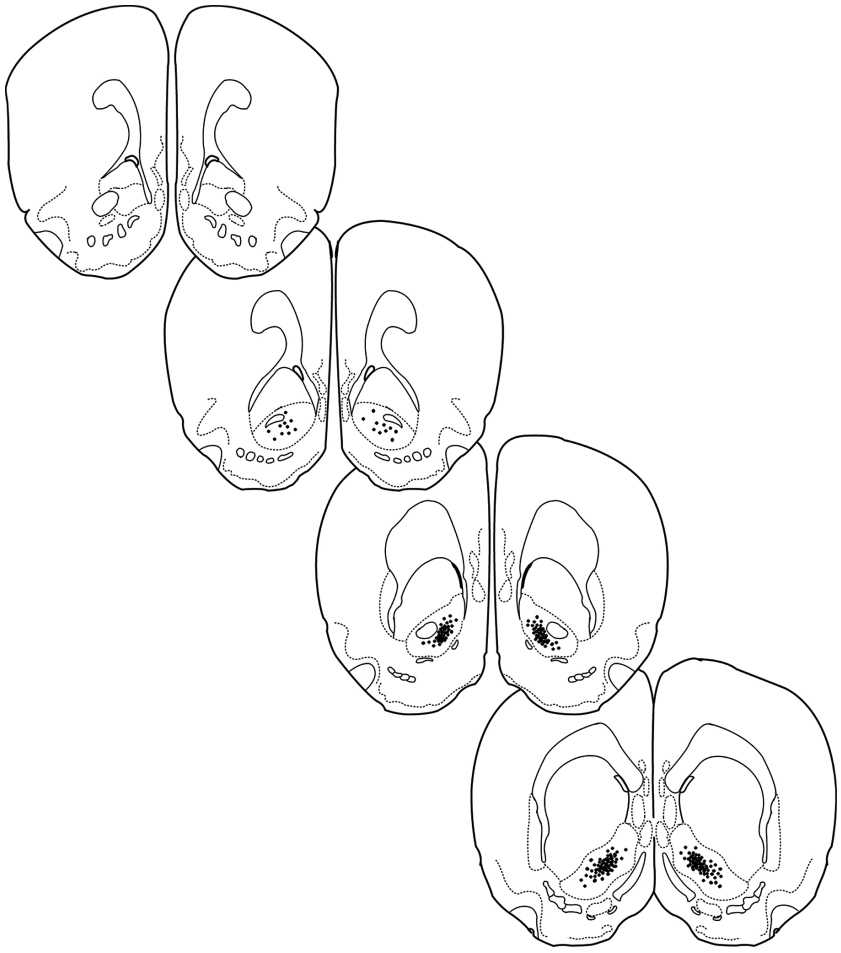

Figure 1: Frontal brain sections showing the location of the injection site.
●: Cannulae placements [20].

Council (Directive 86/609/EEC). Bioethical rules established by the Faculty of Medicine of the National University of Cuyo and by Argentine laws were also considered.

Results

Histology

All animals had a correct cannula placement (Figure 1).

Standard measures

Time spent in the open arm was modified by the treatments ($F_{4,104} = 3.420$, $p < 0.05$). When groups were compared with saline controls, a significant difference was observed in the 2 and 4 µg injected groups ($p < 0.05$). Open arm entries were influenced by treatment ($F_{4,104} = 2.990$, $p < 0.05$). Except at the lower doses, all showed a significant difference when compared with saline controls ($p < 0.05$) (Figure 2).

Ethological measures

The extreme arrivals were modified by drug treatment ($F_{4,104} = 4.401$, $p < 0.01$). Comparing groups, a significant difference was observed between saline and 1 µg ($p < 0.05$). These differences were increased with the higher doses (2 µg and 4 µg, $p < 0.01$), suggesting an incidence of the dose used on the effect. No differences were observed with the other studied parameters.

Discussion

Dizocilpine (present results) produced increases in the time spent in the open arm with the two higher doses. Even

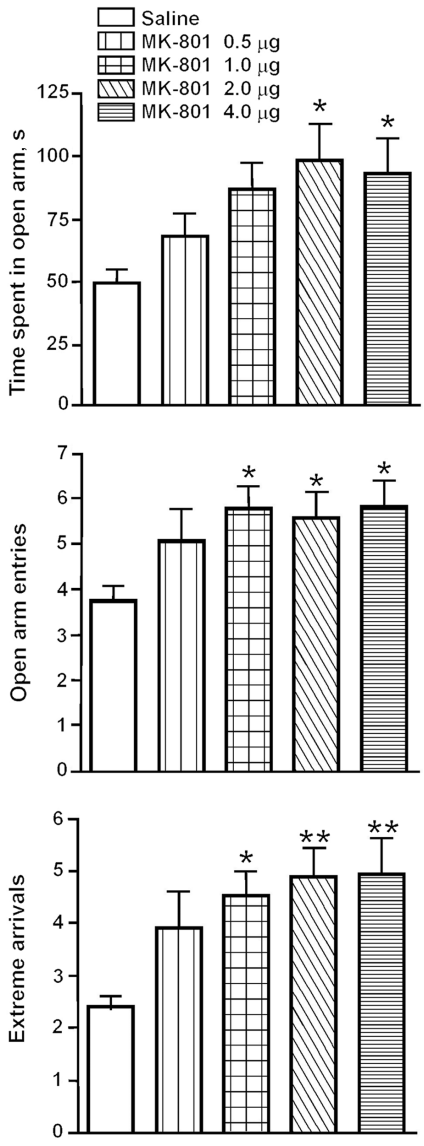

Figure 2: Results from rats given saline (1 μL saline control, n = 25) or dizocilpine (MK-801), 0.5 (n = 14), 1.0 (n = 20), 2.0 (n = 24), and 4 μg/1 μL (n = 22) injections into the NAS.
One-way ANOVA followed by Student-Newman-Keuls test were used. Data are presented as means ± SE (*$p < 0.05$; **$p < 0.01$). Top: time spent in the open arm (s); middle: open arm entries (*$p < 0.05$); bottom: extreme arrivals.

when a dose-response curve was not obtained, a dose-related effect was evident. Time per entry, which we proposed as a specific anxiolytic index independent of motor activity [9–12, 21, 23], was not clearly affected. However, time spent in the open arm is an accepted parameter of anxiolytic effect. The specific indexes of general activity are the number of entries to close arms and the total number of entries [24], and these were not modified in the present conditions. Open arm entries and extreme arrivals were increased by all doses except the lower one.

We previously considered the open arm entries as a possible index of behavioral motor disinhibition, and we attributed it to a striatal blockade of cortical inhibitory inputs [9]. The blockade of these afferences, predominantly glutamatergic, could lead to an increase in open arm entries. It is interesting that this parameter is positive even with lower doses than time spent in the open arm, suggesting a predominantly disinhibitory motor effect at this level.

Extreme arrivals could be related to an increase in locomotor activity but also an anxiolytic-like effect. An addition could be observed in the significance in this parameter, with a higher significance with the doses that gave positive results in both previous parameters (2 and 4 μg). The addition of anxiolytic-like effect and the behavioral disinhibition previously postulated could be exerting the current significant increase. It may be concluded that the effect of dizocilpine is anxiolytic but predominantly disinhibitory when injected within the NAS. It may be a matter of doubt about the selective condition of the anxiolytic-like effect.

Differentially, the behavioral profile observed in our conditions was different injecting AP-7 [(±) 2-amino-7-phosphonoheptanoic acid, a selective NMDA antagonist] or NBQX (2,3-dioxo-6-nitro-1,2,3,4,tetrahydrobenzo-(*f*)quinoxaline-7-sulfonamide disodium, a non-NMDA antagonist) within NAS induced different behavioral patterns. AP-7 induced a pattern characterized by an increase in the time spent in the open arm (two higher doses) but also in time per entry, suggesting a typical anxiolytic effect. Also, open arm entries and extreme arrivals were clearly increased by the middle dose, suggesting behavioral disinhibition. NBQX induced an increase in the time spent in the open arm only with the higher dose, but not in time per entry, and increases in open arm entries but not in extreme arrivals. It may be interpreted as an incomplete anxiolytic pattern. All the evidence strongly suggests that blockade of different sites of the NMDA receptor mediate different behavioral patterns.

In previous studies, we have proposed that the glutamatergic NMDA blockade within NAS resembles all the main schizophrenic symptoms in animal models: positive, negative and cognitive symptoms [9, 10, 13–15], starting from clinical studies [25], and extensively reviewed [3, 4, 26]. We interpreted these observed increases in time spent in the open arm as homologous signs of affective flattening, mimicking this illness condition pharmacologically.

We conclude that the present results obtained with dizocilpine led to a significant decrease in emotionality levels when injected within the NAS. Once again, NAS shows a very relevant role in anxiety processing in the plus maze test. We may also conclude that anxiolytic-like effects do not seem to be here merely explained by

an increase in locomotion. Finally, we have observed that the blockade of different sites of the NMDA receptor induces different behavioral patterns in the same laboratory conditions, which was one of the main rationales of the present study.

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