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Research report

Effects of the inducible nitric oxide synthase inhibitor aminoguanidine in two different rat models of schizophrenia

Anastasios Lafioniatis, Martha A. Orfanidou, Evangelia S. Papadopoulou, Nikolaos Pitsikas*

Department of Pharmacology, School of Medicine, Faculty of Health Sciences, University of Thessaly, Larissa, Greece

HIGHLIGHTS

- Ketamine and apomorphine disrupted recognition memory.
- Ketamine induced social isolation.
- AG reversed the recognition memory deficits described above.
- AG did not attenuate ketamine-induced social isolation.

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ABSTRACT

Several lines evidence indicate that the non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist ketamine and the mixed dopamine (DA) D_1/D_2 receptor agonist apomorphine induce schizophrenia-like symptoms in rodents, including memory impairments and social withdrawal. Nitric oxide (NO) has been proposed to act as an intracellular messenger in the brain and its overproduction is associated with schizophrenia. The current study was designed to investigate the ability of the inducible NO synthase (iNOS) inhibitor aminoguanidine (AG) to counteract schizophrenia-like behavioural deficits produced by ketamine and apomorphine in rats. The efficacy of AG to antagonize extinction of recognition memory, ketamine and apomorphine-induced recognition memory impairments was tested utilizing the novel object recognition task (NORT). Further, the efficacy of AG to attenuate ketamine-induced social withdrawal was examined in the social interaction test. AG (25 and 50 mg/kg) antagonized extinction of recognition memory and reversed ketamine (3 mg/kg) and apomorphine (1 mg/kg)-induced recognition memory deficits. In contrast, AG (50 and 100 mg/kg) did not counteract the ketamine (8 mg/kg)-induced social isolation. The present data show that the iNOS inhibitor AG counteracted extinction of recognition memory and reversed recognition memory deficits produced by dysfunction of the glutamatergic and the dopaminergic (DAergic) system in rats. Therefore, AG may be efficacious in attenuating memory impairments often observed in schizophrenia patients.

1. Introduction

Schizophrenia is a severe psychiatric disorder that affects up to 1% of the population in the world [44]. Schizophrenia symptoms, can be divided in three categories: positive symptoms (i.e., hallucinations, delusions, disordered thought processing), negative symptoms (social withdrawal, anhedonia, avolition) and cognitive impairments (attentional and memory deficits) [26].

Consistent experimental evidence indicates that the glutamatergic system, especially *N*-methyl-D-aspartate (NMDA) receptors, might be altered in schizophrenia [16]. In particular, non-competitive NMDA receptor antagonists like phencyclidine (PCP), MK-801 or ketamine were found to induce behavioural symptoms in healthy subjects mimicking both positive and negative symptoms of this disease [34,40] and exacerbate symptoms in schizophrenic individuals [41,43]. Additionally, these NMDA receptor antagonists induce schizophrenia-like symptoms, including cognitive deficits, in rodents [9,51,71,72].

* Corresponding author at: Department of Pharmacology, School of Medicine, Faculty of Health Sciences, University of Thessaly, Biopolis, Panepistimiou 3, Larissa 415-00, Greece.
E-mail address: npitsikas@med.uth.gr (N. Pitsikas).

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Several lines of evidence suggest that the dopaminergic (DAergic) system might be compromised in schizophrenia. Deficits in performance in various cognitive tasks have been described in this disorder, linked to decreased prefrontal dopamine (DA) functioning [33]. Evidence from both preclinical and clinical research using pharmacological stimulation of DA receptors proposes that either too little or too much DA stimulation disturbs cognition [14,29,30,73].

Traditional neuroleptics have demonstrated a certain efficacy in the treatment of the positive symptoms of schizophrenia, however their efficacy is weak in the treatment of negative symptoms and cognitive deficits of this disease [25].

Nitric oxide (NO), a soluble, short-lived and freely diffusible gas, is an important intracellular messenger in the brain [27]. NO is originated by the conversion of L-arginine to L-citrulline, with the efflux of NO. The enzyme responsible for the generation of NO is NO synthase (NOS). Three NOS isoforms encoded on different distinct genes were described: Neuronal NOS (nNOS, NOS type I) which is the isoform found in the neuronal tissue, inducible NOS (iNOS, NOS type II) which is the isoform who's synthesis is induced by various pro-inflammatory factors (cytokines or endotoxin) and endothelial (eNOS, NOS type III) which is the isoform expressed in the endothelium [10].

NO plays an important role in cognition [56] and is involved in the mechanisms of synaptic plasticity in the hippocampus [31,52]. Consistent experimental evidence indicates the involvement of NO in schizophrenia. Both underproduction and overproduction of NO are associated to schizophrenia although the direction of abnormalities is still uncertain [5]. Reportedly, an abnormal distribution of nitrergic neurons in the frontal and temporal lobes of schizophrenia patients has been observed [1]. Further, experimental evidence suggests that polymorphisms in the nNOS gene are linked to schizophrenia and prefrontal cortex (PFC) function in schizophrenics [58]. Contrasting results were reported however, regarding the levels of NO metabolites in the serum of schizophrenia patients. Either high [68,79] or low [57] concentrations of NO metabolites have been reported.

Because both alterations in the synaptic organization of the brain [59] and neurotransmitter impairments [15] are of critical importance for schizophrenia, overproduction of NO might contribute to disturbed neurocircuitry in this disorder. It has been reported that exaggerated NO concentrations are related to neuronal damage [18], mitochondrial dysfunction [17] and impairment of the NMDA-receptor mediated neurotransmission [13]. Altogether, these findings indicate that reduced NOS activity in schizophrenia may be neuroprotective. Thus, the development of NOS inhibitors might be a potential therapeutic tool for schizophrenia [4].

Consistent preclinical research has demonstrated that various non-selective or nNOS inhibitors attenuated psychotomimetic effects, cognitive deficits and social isolation caused both by glutamate hypofunction [9,35–77] and DAergic hyperactivity [30,61,62]. There is scant information, however, regarding the role of iNOS inhibitors in this psychiatric disorder. In this context, it has recently been reported that the iNOS inhibitors aminoguanidine (AG) and EGCG attenuated NMDA receptor antagonist-induced hypermotility, ataxia and excessive glutamate release from medial PFC (mPFC) in the rat [3]. At the moment, it remains uncertain whether or not iNOS inhibitors are able to reverse cognitive deficits and social isolation produced by dysfunction of the glutamatergic or DAergic system in animals. The present research was designed to assess this issue.

Based on the above described findings, the first purpose of the current work was to investigate the efficacy of AG, a selective inhibitor of iNOS [8], in counteracting the extinction of recognition memory in the rat. Subsequently, we aimed to test the ability of this iNOS inhibitor in reversing ketamine and apomorphine-induced recognition memory deficits. Recognition memory is a type of memory that is impaired in schizophrenic patients [11,22] and disrupted by both ketamine and apomorphine in healthy volunteers [48,49] and rats [9,30]. For this purpose, the novel object recognition task (NORT) was used [23]. Finally, the efficiency of AG to attenuate the social withdrawal produced by ketamine in a social interaction test was investigated [39]. Social interaction deficits produced by treatment with NMDA receptor antagonists resemble the negative symptoms of schizophrenia [39,63].

2. Material and methods

2.1. Subjects

Independent groups of naive male (3-month-old) Wistar rats (Hellenic Pasteur Institute, Athens, Greece) weighing 250–300 g were used. The animals were housed in Makrolon cages (47.5 cm length × 20.5 cm height × 27 cm width), three per cage, in a regulated environment (21 ± 1 °C; 50–55% relative humidity; 12-h/12-h light/dark cycle, lights on at 07.00 h) with free access to food and water.

The procedures that involved animals and their care were in accordance with international guidelines and national (Animal Act, P.D. 160/91) 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. Novel object recognition task (NORT)

The test apparatus consisted of a dark open box made of Plexiglas (80 cm length × 50 cm height × 60 cm width) that was illuminated by a 60-W light suspended 60 cm above the box. The light intensity was equal in the different parts of the apparatus. The objects to be discriminated (in triplicate) made of glass, plastic, or metal, were in three different shapes: metallic cubes, glass pyramids and plastic cylinders 7 cm high; and could not be displaced by rats.

NORT was performed as described previously [9]. Briefly, during the week before the test, the animals were handled twice a day for 3 consecutive days. Before testing, the rats were allowed to explore the apparatus for 2 min for 3 consecutive days. During testing, a session that consisted of two 2-min trials was conducted. During the "sample" trial (T1), two identical samples (objects) were placed in two opposite corners of the apparatus in a random fashion, 10 cm from the side walls. A rat was placed in the middle of the apparatus and allowed to explore the two identical objects. After T1, the rat returned to its home cage and an intertrial interval (ITI) followed. Subsequently, the "choice" trial (T2) was performed. During T2, a novel object replaced one of the objects presented during T1. Accordingly, the rats were re-exposed to two objects: a copy of the familiar (F) object and the novel (N) object. All combinations and locations of the objects were counterbalanced to reduce potential bias caused by preference for particular locations or objects. Exploration was defined as follows: directing the nose toward the object at a distance of 2 cm or less and/or touching the object with the nose. Turning around or sitting on the object was not considered exploratory behaviour. The time spent by the rats exploring each object during T1 and T2 was manually recorded with a stopwatch. Based on this measure, a series of variables was then calculated: the total time spent exploring the two identical objects in T1 and the time spent exploring the two different objects, (F) and (N) in T2. The discrimination between (F) and the (N) during T2 was measured by comparing the time spent exploring the familiar object with the time spent exploring the novel object. Because this time