Research report

Nicotinic receptors and lurasidone-mediated reversal of phencyclidine-induced deficit in novel object recognition

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HIGHLIGHTS

- Mecamylamine, a non-selective nAChR antagonist, induced a NOR deficit in normal rats.
- A-85380, a selective $\alpha_4\beta_2*$ nAChR agonist, reversed PCP-induced NOR deficit.
- PNU-282987, a selective α_7 nAChR agonist, reversed PCP-induced NOR deficit.
- The reversal effect of lurasidone was blocked by mecamylamine.
- Sub-effective dose of A-85380 and PNU-282987 potentiated the effect of lurasidone.

ARTICLE INFO

ABSTRACT

Background: Enhancement of cholinergic function via nicotinic acetylcholine (ACh) receptor (nAChR) agonism is a potential approach for the treatment of cognitive impairment associated with schizophrenia (CIAS). Some atypical antipsychotic drugs (AAPDs) enhance ACh release in rodent brain, indirectly stimulating these receptors. Here, we elucidate which nAChR subtypes mediate novel object recognition (NOR) in normal rats and contribute to the ability of the AAPD, lurasidone, to improve the NOR deficit in sub-chronic (sc) phencyclidine (PCP)-treated rats, a model for CIAS.
Methods: The ability of lurasidone and nAChR ligands to reverse the scPCP-induced deficit in NOR was assessed in female, Long-Evans rats.
Results: The broad acting nAChR antagonist, mecamylamine (MEC), induced a NOR deficit in normal rats. The NOR deficit secondary to scPCP was reversed by either selective $\alpha_4\beta_2*$ nAChR agonism (A-85380) or α_7 nAChRs agonism (PNU-282987); these effects were blocked by DHβE and MLA, selective antagonists of $\alpha_4\beta_2*$ and α_7 nAChR, respectively. The ability of lurasidone to reverse the scPCP-induced NOR deficit was blocked by MEC, but not MLA or DHβE. However, sub-effective doses (SED) of either A-85380 or PNU-282987 potentiated the ability of SED lurasidone to reverse the scPCP-induced NOR deficit.
Conclusions: These results identify both $\alpha_4\beta_2*$ and α_7 nAChRs as candidates for enhancing the ability of lurasidone and other AAPDs, which increase the release of ACh, to improve CIAS.

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1. Introduction

Cognitive impairment associated with schizophrenia (CIAS) is one of the most important factors leading to poor functional outcome in schizophrenia, making development of more efficacious treatments for CIAS a high priority [1,2]. Atypical antipsychotic drugs (AAPDs), the most widely used agents to treat positive symptoms, are also effective in attenuating impairments in declarative memory and other cognitive domains in some patients with schizophrenia [3,4]. Most clinical trials and all meta-analyses of those data show superiority for AAPDs over typical APDs in improving specific cognitive domains in patients with schizophrenia [5,6].

Animal models of CIAS may be of value to study the pathophysiology of, and to develop treatment for, CIAS [7]. The novel object recognition (NOR) test in rodents is a non-rewarded, ethologically relevant paradigm, that has been widely used to model human declarative memory [8]. Several brain regions, especially the hippocampus (HIP) and the prefrontal cortex (PFC), have been implicated in both rodent NOR and the analogous human declarative memory [9]. It has been reported that acute treatment with an N-methyl-D-aspartate receptor (NMDAR) antagonist [e.g., phencyclidine (PCP)], produces NOR deficits [7,10]. Although AAPDs have been found to reverse the scPCP-induced NOR deficits in rodents [11,12], typical APDs are ineffective [7,10,11]. Lurasidone is an AAPD with 5-hydroxytryptamine (5-HT)$_{2A}$, 5-HT$_7$, dopamine (DA) D$_2$ antagonist, and 5-HT$_{1A}$ receptor partial agonist properties [13]. Lurasidone significantly increases DA, acetylcholine (ACh), and glutamate (Glu) efflux in rat medial PFC (mPFC), and also produces a small increase in DA and Glu efflux in the nucleus accumbens (NAc) [14]. Lurasidone also significantly increases extracellular ACh in HIP in rats (in preparation).

Cholinergic mechanisms play a critical role in spatial representation in the HIP, possibly through GABAergic influences which regulate theta rhythms [15], which are known to be abnormal in schizophrenia [16]. Nicotinic acetylcholine receptors (nAChR) fine tune GABAergic interneuron activity which is essential to enable the participation of pyramidal neurons in learning and memory [17]. However, EVP-6124, a high-affinity α_7 nAChR partial agonist, does not significantly affect GABA efflux in mPFC or NAc, but does increase DA, Glu and ACh efflux in the mPFC and DA in the NAc [18]. Pretreatment with the α_7 nAChR antagonist, methyllycaconitine (MLA), significantly blocked cortical DA and Glu efflux induced by EVP-6124, suggesting that these effects are due to α_7 nAChR agonism.

Abnormalities in nAChRs have been reported in schizophrenia and likely contribute to some aspects of CIAS [19]. Further, nAChR antagonists given to healthy volunteers induce cognitive impairments [20,21]. Consistent with these findings, nAChR agonists enhance cognition in normal subjects [22,23]. Increasing ACh signaling with nAChR agonists, as well as acetylcholinesterase (AChE) inhibitors, improves attentional and memory-related deficits, in some studies of schizophrenia [24,25]. Varenicline, an $\alpha_4\beta_2$* partial agonist and α_7 agonist, significantly improves performance in multiple cognitive domains when given with APDs to schizophrenia patients [26]. Similar results have been reported with EVP-6124 [27,28]. Consistent with these clinical findings, nAChR agonists and AChE inhibitors enhance cognitive function [23,29] and α_7 nAChR agonists, such as WYE-103914, reverse the NMDAR antagonist-induced NOR deficits in rodents [30]. These pre-clinical studies suggest that deficient nAChR signaling may underlie, at least in part, CIAS.

The aim of the present study was to determine: (1) the nAChR subtypes that contribute to NOR in normal rats; and (2) the role of nAChR in the ability of lurasidone to improve the scPCP-induced deficit in NOR. First, we assessed whether a non-subtype selective nAChR antagonist, MEC, a selective β_2* nAChR antagonist, dihydro-β-erythroidine hydrobromide (DHβE), or MLA, impairs NOR in normal rats. We then determined the ability of the $\alpha_4\beta_2$* nAChR agonist, A-85380, and the α_7 nAChR agonist, PNU-282987, to reverse the scPCP-induced deficit in NOR. Next, we determined whether nAChR antagonism was able to prevent the restorative effects of lurasidone. Lastly, we examined whether co-administration with A-85380 or PNU-282987 was sufficient to potentiate the ability of lurasidone to reverse the scPCP-induced deficit.

2. Material and methods

2.1. Animals

Female, Long–Evans rats ($n = 204$; 8–9 weeks old; Harlan Laboratories, IN, USA) were used for the NOR testing. All rats were housed in groups of two or three on a 14 h/10 h light/dark cycle with free access to food and water. All procedures were performed in accordance with the Institutional Care and Use Committees of Northwestern University.

2.2. Drugs

Lurasidone was provided by Sumitomo Dainippon Pharma Co., Ltd. (Osaka, Japan). 5-Iodo-A-85380 dihydrochloride hydrate (A-85380), dihydro-β-erythroidine hydrobromide (DHβE), PNU-282987, and methyllycaconitine citrate (MLA) were obtained from Tocris bioscience (MN, USA). Mecamylamine hydrochloride (MEC) was obtained from Sigma-Aldrich (MO, USA). Phencyclidine (PCP) was supplied as a generous gift from the National Institute of Drug Abuse. Lurasidone and PNU-282987 were dissolved in 0.5% methylcellulose and 0.2% Tween80. MEC was dissolved in 5% dimethyl sulfoxide (DMSO). PCP, A-85380, DHβE, and MLA were dissolved in sterile distilled water. All drugs or vehicle were administered intraperitoneally (i.p.), at a volume of 1 mL/kg body weight. DHβE, MLA and MEC were administrated 45 min before the NOR test (Figs. 2–4) and all other drugs were administrated 30 min before the NOR test. For treatment with agonists and antagonists, doses were determined from previous studies to minimize non-selective activity [31–33].

2.3. Drug treatment and NOR testing

All rats were habituated for 1 hour to the plexiglass NOR apparatus ($52 \times 52 \times 31$ cm) for three consecutive days prior to the first NOR test. On each day of testing, rats were given an additonal 3-min habituation phase. Rats were then given two 3-min trials (an acquisition and a retention trial), separated by a 1-min intertrial interval during which they were returned to the home cage. During the acquisition trial, the animals were allowed to explore two identical objects. During the retention trial, the animals explored a familiar object from the acquisition trial and a novel object. Behavior was recorded on video for subsequent scoring of time spent exploring each object using blind procedures. Object exploration was defined by animal's licking, sniffing, or touching the object with the forepaws while sniffing. Object exploration (s) was recorded manually by the use of two stopwatches. The discrimination index (DI) [(time spent exploring the novel object—time spent exploring the familiar object)/(total time spent exploring both objects)] was calculated for the retention trials.

Rats were randomly assigned to two treatment groups: 8–9 were treated with vehicle (saline, ip) and the remainder were treated with PCP (2 mg/kg, ip) twice daily for 7 days. Subsequently, animals were given a 7-day washout period prior to NOR testing [11,34]. Each rat was tested four times in the NOR paradigm with a 7-day washout period between each of the test sessions to reduce