Behavioural Brain Research 273 (2014) 166–176

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Asenapine sensitization from adolescence to adulthood and its potential molecular basis

Qing Shu[a,c,1], Rongyin Qin[b,c,d,1], Yingzhu Chen[b], Gang Hu[a,**], Ming Li[c,*]

[a] Jiangsu Key Laboratory of Neurodegeneration, Department of Pharmacology, Nanjing Medical University, Nanjing, Jiangsu, PR China
[b] Department of Neurology, The Clinical Medical College of Yangzhou University, Yangzhou, Jiangsu, PR China
[c] Department of Psychology, University of Nebraska-Lincoln, USA
[d] Department of Neurology, Changzhou No. 2 People's Hospital, Nanjing Medical University, Changzhou, Jiangsu, PR China

HIGHLIGHTS

- Five days of asenapine treatment in adolescence caused a sensitization in adulthood.
- Asenapine sensitization was demonstrated in a conditioned avoidance response test.
- Asenapine sensitization was demonstrated in a phencyclidine-induced hyperlocomotion test.
- Adolescent asenapine treatment did not cause a long-term change in the levels of BDNF, D_2 receptor and ΔFosB.

ARTICLE INFO

Article history:
Received 31 March 2014
Received in revised form 22 July 2014
Accepted 25 July 2014
Available online 2 August 2014

Keywords:
Asenapine
Conditioned avoidance response
Phencyclidine
BDNF
Dopamine D_2 receptor
ΔFosB

ABSTRACT

Asenapine is a new antipsychotic drug that induces a long-lasting behavioral sensitization in adult rats. The present study investigated the developmental impacts of adolescent asenapine treatment on drug sensitivity and on 3 proteins implicated in the action of antipsychotic drugs (i.e. brain-derived neurotrophic factor (BDNF), dopamine D_2 receptor, and ΔFosB) in adulthood. Male adolescent Sprague–Dawley rats (postnatal days, P 43–48) were first treated with asenapine (0.05, 0.10 or 0.20 mg/kg, sc) and tested in the conditioned avoidance or PCP (2.0 mg/kg, sc)-induced hyperlocomotion tasks for 5 days. After they became adults (~P 76), asenapine sensitization was assessed in a single avoidance or PCP-induced hyperlocomotion challenge test with all rats being injected with asenapine (0.10 mg/kg, sc). Rats were then sacrificed 1 day later and BDNF, D_2 and ΔFosB in the prefrontal cortex, striatum and hippocampus were examined using Western blotting. In adolescence, repeated asenapine treatment produced a persistent and dose-dependent inhibition of avoidance response, spontaneous motor activity and PCP-induced hyperlocomotion. In the asenapine challenge test, adult rats treated with asenapine (0.10 and 0.20 mg/kg) in adolescence made significantly fewer avoidance responses and showed a stronger inhibition of spontaneous motor activity than those previously treated with saline. However, no group difference in the levels of BDNF, D_2 and ΔFosB expression was found. These findings suggest that although adolescent asenapine treatment for a short period of time induces a robust behavioral sensitization that persists into adulthood, such a long-term effect is not likely to be mediated by BDNF, D_2 and ΔFosB.

1. Introduction

Antipsychotic treatment in children and adolescents has increased dramatically in recent decades. Epidemiological surveys conducted in many countries (e.g. UK, US, Germany, Netherlands) indicate a 2–6-fold increase in the number of prescribed antipsychotic drugs for young patients (≤20 years) between the 1990s and the mid-2000s [1–3]. As adolescence is a unique period when the brain undergoes dramatic re-organization and frontal maturation, it is conceivable that antipsychotic exposure during this period will alter brain development and behavioral function in the

* Corresponding author at: Department of Psychology, University of Nebraska-Lincoln, 238 Burnett Hall, Lincoln, NE 68588-0308, USA. Tel.: +1 402 472 3144.
** Corresponding author at: Jiangsu Key Laboratory of Neurodegeneration, Department of Pharmacology, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu 210029, PR China. Tel.: +86 25 86863108; fax: +86 25 86863108.
E-mail addresses: ghu@njmu.edu.cn (G. Hu), mli@unl.edu (M. Li).
[1] These authors contribute equally to this work.

http://dx.doi.org/10.1016/j.bbr.2014.07.042
0166-4328/

long run. Recent preclinical studies suggest that this is indeed the case. For example, periadolescent exposure to antipsychotic drugs such as olanzapine, risperidone and clozapine is found to alter various neuroreceptors, including dopamine D_1, D_2 and D_4 receptors [4,5], serotonin 5-HT_{1A} and 5-HT_{2A} receptors [6], and ionotropic NMDA and AMPA glutamatergic receptors [7]. Adolescent antipsychotic treatment is also shown to enhance rodents' sensitivity to amphetamine [5], impair their working memory in a delayed nonmatch to sample test, delay the extinction process of shock-induced fear memory in adulthood [8], and prevent the development of various psychosis-like behaviors [9–12].

Our research on the long-term effects of antipsychotic treatment on behavioral and brain functions throughout development has focused on the drug-induced alterations in drug sensitivity from adolescence to adulthood [13–16]. As is often the case, repeated administration of a psychotropic drug results in either an increase or decrease of a particular behavioral effect of the drug, termed *sensitization* and *tolerance*, respectively [17]. Our previous antipsychotic work on adult rats (>70 days old) identify two similar behavioral phenomena, which are termed antipsychotic sensitization and tolerance [18–23]. Using two distinct behavioral tests of antipsychotic activity: conditioned avoidance response (CAR) and PCP-induced hyperlocomotion, we showed that repeated administration of haloperidol, olanzapine, asenapine or risperidone daily for 5–7 days in adult rats progressively increases the drug's efficacy to inhibit avoidance responding and PCP-induced hyperlocomotion over time (a within-subjects index of sensitization). A few days later, when all rats are given a challenge dose of these drugs, they often make significantly *fewer* avoidance responses and exhibit *lower* PCP-induced hyperlocomotion than those that are treated with these drugs for the first time (a between-subjects index of sensitization). In contrast, repeated administration of clozapine causes a decrease in its behavioral efficacy in these tests, indicating a tolerance effect. In addition, our previous studies also indicate that antipsychotic sensitization that can last up to 50 days [19], and are likely mediated by dopamine D_2 and 5-HT_{2A} receptor-related neural plasticity [23].

Recently we expanded our antipsychotic sensitization and tolerance work into the adolescent period. We have demonstrated that adolescent antipsychotic treatment could induce behavioral sensitization and tolerance that maintain into adulthood. Adolescent treatment of olanzapine or risperidone causes a sensitization effect, whereas clozapine treatment in adolescence causes a tolerance in adulthood [13–15,24]. Rats treated with only 5 days of olanzapine or risperidone in adolescence showed a stronger inhibition of CAR and PCP-induced motor activity than those treated with vehicle when all rats were challenged with the same antipsychotic drug in adulthood, whereas those treated with clozapine for 5 days in adolescence showed a weaker inhibition than the vehicle rats. In addition, adolescent risperidone treatment even altered adulthood responsiveness to other atypical drugs (e.g. olanzapine and clozapine) [15]. Collectively, these findings provide strong evidence that antipsychotic treatment in adolescence can induce a long-term change in drug responsiveness that persists into adulthood.

The primary goal of the present study was to determine the generality of this observation by examining whether asenapine, a newer atypical antipsychotic drug with a distinctive receptor binding profile from other atypical antipsychotic drugs [25,26], would also cause a sensitization effect that persists from adolescence into adulthood. In addition, we attempted to identify the possible molecular mechanisms underlying such a long-term behavioral effect. Given that adolescent antipsychotic treatment is known to increase dopamine D_2 receptors in certain forebrain regions [4,5], and repeated antipsychotic treatment is shown to induce a robust long-term change in BDNF and ΔFosB (a transcription factor), two important biomarkers involved in brain plasticity and the action of chronic antipsychotic treatment [27–31], we focused our attention on the D_2, BDNF and ΔFosB levels in the prefrontal cortex (PFC), striatum, and hippocampus in adult rats that had received either asenapine or vehicle treatment in adolescence. These brain areas have been implicated in antipsychotic action and the neuropathology of schizophrenia [32]. Our hypothesis was that adolescent asenapine treatment would induce a sensitization effect that persists into adulthood. In addition, we expected that adolescent asenapine treatment would also cause long-lasting changes in the expression of D_2, BDNF and ΔFosB levels parallel to the behavioral sensitization.

2. Materials and methods

2.1. Animals

Adolescent male Sprague–Dawley rats from Charles River (Portage, MI; postnatal days, P 25–27 or P 35–37 upon arrival, averaged age were assumed to be ∼P 26 or ∼P 36) were used. They were housed two per cage, in 48.3 cm × 26.7 cm × 20.3 cm transparent polycarbonate cages under 12-h light/dark conditions (light on between 6:30 am and 6:30 pm). Room temperature was maintained at 22 ± 1 °C with a relative humidity of 45–60%. Food and water was available *ad libitum*. Rats were allowed at least 5 days of habituation to the animal facility before being used in experiments (∼P 31 or ∼P 41). All behavioral tests took place between 9 am and 5 pm in the light cycle. The experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln.

2.2. Drugs and choice of doses

Asenapine maleate (ASE, a gift from the NIMH drug supply program) was dissolved in 0.9% saline. Doses of asenapine (0.05, 0.10 and 0.20 mg/kg) were determined on the basis of our literature review showing that this dose range of asenapine causes a dose-dependent suppression of CAR but does not cause severe motor impairment [33,34]. These doses were also chosen on the basis of our recent studies showing that asenapine at these doses induces a dose-dependent and long-lasting sensitization in adult rats in the CAR test [18,19]. Phencyclidine hydrochloride (PCP, gift from the NIDA Chemical Synthesis and Drug Supply Program) was dissolved in 0.9% saline and tested at 2.00 mg/kg. All drugs were administrated subcutaneously (sc) at 1.00 ml/kg.

2.3. Two-way avoidance conditioning apparatus and ultrasonic vocalization (USV) apparatus

Eight identical two-way shuttle boxes custom designed and manufactured by Med Associates (St. Albans, VT) were used. Each box was housed in a ventilated, sound-insulated isolation cubicle (96.52 cm W × 35.56 cm D × 63.5 cm H). Each box was 64 cm long, 30 cm high (from grid floor), and 24 cm wide, and was divided into two equal-sized compartments by a partition with an arch style doorway (15 cm high × 9 cm wide at base). A barrier (4 cm high) was placed between the two compartments, so the rats had to jump from one compartment to the other. The grid floor consisted of 40 stainless-steel rods with a diameter of 0.48 cm, spaced 1.6 cm apart center to center, through which a scrambled footshock (unconditioned stimulus, US, 0.8 mA, maximum duration: 5 s) was delivered by a constant current shock generator (Model ENV-410B) and scrambler (Model ENV-412). The rat location and crossings between compartments were monitored by a set of 16 photobeams (ENV-256-8P) affixed at the bottom of the box (3.5 cm above the grid floor). Illumination was provided by two