Neuropharmacology 108 (2016) 264–274

Contents lists available at ScienceDirect

Neuropharmacology

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

Risperidone induces long-lasting changes in the conditioned avoidance response and accumbal gene expression selectively in animals treated as adolescents

Aung Aung Kywe Moe [a], Nyoman D. Kurniawan [b], Suzanne Alexander [a, c], Xiaoying Cui [a], Thomas H.J. Burne [a, c], Darryl W. Eyles [a, c, *]

[a] Queensland Brain Institute, The University of Queensland, Australia
[b] Centre for Advanced Imaging, The University of Queensland, Australia
[c] Queensland Centre for Mental Health Research, Australia

ARTICLE INFO

Article history:
Received 13 October 2015
Received in revised form
28 January 2016
Accepted 25 April 2016
Available online 26 April 2016

Keywords:
Antipsychotic drugs
Animal model
Adolescent
Conditioned avoidance response
Long-term effects
$5HT_{2A}$ receptor
MRI

ABSTRACT

Adolescence is a period of dynamic remodeling and maturation in the brain. Exposure to psychotropic drugs during adolescence can potentially alter neural maturation in the adolescent brain subsequently altering neural function at maturity. In this regard, antipsychotic drugs (APDs) are important given a notable global increase in prescription of these APDs to adolescents for a variety of behavioural symptoms and conditions over the past twenty years. However, there is a paucity of data on the long-term consequences of APDs on the adolescent brain. In this preclinical study, we have examined whether the adolescent brain is more susceptible than the adult brain to long-term neural changes induced by risperidone, which is the APD most frequently prescribed to adolescents. Rats were chronically treated (21 days) with 1.3 mg/kg/day risperidone or vehicle either as adolescents (postnatal day (PND) 36–56)) or adults (PND80-100). Behaviour was assessed using the well-described suppression of the conditioned avoidance response (CAR) by APDs. We examined CAR after all animals had reached maturity (PND127). We show that mature rats treated with risperidone as adolescents had increased CAR suppression compared to adults when rechallenged with this same drug. In the nucleus accumbens, significant downregulation of serotonergic $5HT_{2A}$ receptors and catechol-o-methyl transferase mRNA levels was observed only in the adolescent treated animals. Impaired $5HT_{2A}$ receptor signaling may explain the increased CAR suppression observed in rats treated with risperidone as adolescents. Magnetic resonance imaging (MRI), however, did not detect any risperidone-induced long-term brain structural change at maturity. These findings confirm that APD administration during adolescence may produce long-term behavioural and neurochemical alterations.

1. Introduction

Adolescence is a sensitive postnatal developmental period in which the brain is undergoing major maturation and remodeling processes. The World Health Organization has defined the adolescent period to be between 9 and 20 years of age. During this period, changes in regional brain structure (Giedd et al., 1999; Gogtay et al., 2004), dopaminergic systems (Haycock et al., 2003; Seeman et al., 1987), serotoninergic systems (Chambers et al., 2003; Takeuchi et al., 2000) and ultrastructure (Huttenlocher, 1979; Petanjek et al., 2011) have all been documented. In rats, the adolescent period spans from postnatal day (PND) 28 to PND 60, which is roughly equivalent to the human adolescent period (Andersen and Navalta, 2004; Brenhouse and Andersen, 2011; Burke and Miczek, 2014; McCutcheon and Marinelli, 2009; Spear, 2007). During this period, the rat brain also undergoes important maturation changes in dopaminergic (Andersen et al., 2000; Matthews et al., 2013; McCutcheon et al., 2012; McCutcheon and Marinelli, 2009; Tarazi et al., 1998, 1999; Teicher et al., 1995) and gamma-amino butyric acid (GABA)-ergic systems (Caballero et al., 2014). Given the relevance of these neurotransmitter systems to psychiatric conditions, adolescence is recognized as a period of vulnerability (Paus et al.,

* Corresponding author. Room 529, QBI building (#79), The University of Queensland, Brisbane 4072, Queensland, Australia.
E-mail address: d.eyles@uq.edu.au (D.W. Eyles).

http://dx.doi.org/10.1016/j.neuropharm.2016.04.035
0028-3908/

2008). In addition, it has been hypothesized that psychopharmacological agents, when given in adolescence, can alter the maturation changes in the adolescent brain, subsequently inducing persistent changes in neural function in adulthood (Andersen, 2003; Andersen and Navalta, 2004; Fuhrmann et al., 2015; Spear, 2007).

Exposure to antipsychotic drugs (APDs) in adolescence is important in this regard. Notably, a pronounced rise in APD prescription has been observed in adolescent populations over the past two decades across multiple countries, for example, in the United States (Olfson et al., 2006, 2012), Canada (Ronsley et al., 2013), the Netherlands (Kalverdijk et al., 2008), China (Song and Guo, 2013), Australia (Hollingworth et al., 2013) and Israel (Gilat et al., 2011). Atypical APDs, such as olanzapine and risperidone, are more frequently prescribed to adolescents compared to typical APDs (Hollingworth et al., 2013; Olfson et al., 2006, 2012). These studies also show that male adolescent patients are more frequently prescribed with APDs than female adolescent patients. APDs target multiple neurotransmitter systems (for example, see (Kapur et al., 2000)) and can induce structural alterations in certain brain regions (Gur et al., 1998; Lieberman et al., 2005). Typical APDs, for example, haloperidol, target mainly at the dopaminergic receptors whereas atypical APDs such as risperidone show a wide range of affinities for neurotransmitter receptors including dopaminergic, serotonergic and muscarinic receptors (See reviews (Lieberman et al., 2008; Miyamoto et al., 2005)). Given that neural systems targeted by APDs are still maturing in adolescence, a high plausibility exists that such systems may be permanently affected by adolescent APD exposure. However, long-term effects of chronic APD treatment on the immature adolescent brains are poorly understood. This state of affairs is receiving increasing attention and concerns continue to be raised regarding the safety of adolescent APD use (Arango et al., 2004; Ben Amor, 2012; Correll, 2008; McKinney and Renk, 2011; Vitiello et al., 2009). Comprehensive preclinical studies will help to both clarify the long-term neurobiological consequences of such exposures and begin to address whether such concerns are warranted (Vitiello et al., 2009).

Where they exist, most preclinical studies have concentrated on *short-term* consequences i.e. the effects proximal to the termination of APD exposure or have used *adult* subjects. Only very recently have some researchers started to document persistent neural outcomes in animals exposed to APDs as adolescents. For instance, olanzapine treatment in adolescence has now been reported to induce long-lasting alterations in adult reward behaviour and dopamine neurotransmission in the nucleus accumbens (NAc) (Vinish et al., 2013) along with producing deficits in adult working memory and fear conditioning (Milstein et al., 2013) and decreased GABA and glutamate levels in the adult NAc (Xu et al., 2015). In addition, adolescent olanzapine exposure has also been reported to alter adult cognitive performance in novel object recognition (Llorente-Berzal et al., 2012). APDs are well known to suppress conditioned avoidance responses (CAR) (Wadenberg and Hicks, 1999). APD-induced sensitization of this suppression in adulthood has also been reported with adolescent exposure to risperidone (Qiao et al., 2014), olanzapine (Qiao et al., 2013), asenapine (Shu et al., 2014) and haloperidol (Gao and Li, 2014). Nevertheless, it remains unclear whether the long-term changes in behaviour and neurochemistry observed in these studies are restricted to APD exposure in adolescence due to the lack of appropriate comparison age group treated with the same APD regimen. These experimental shortcomings i.e. importance of comparison age groups in adolescent exposure studies have previously been highlighted (Fuhrmann et al., 2015; Spear, 2007).

The short-term effects of the same APD treatments on neuroreceptors also differ between adolescents and adults. This has been examined in dopaminergic receptors (Moran-Gates et al., 2007), glutamatergic receptors (Choi et al., 2009) and serotonergic receptors (Choi et al., 2010). All these studies addressed these effects at 24 h after the last treatment. For example, 24 h after chronic 21-day risperidone treatment in adolescents, upregulation of D1 receptors in NAc and striatum and of $5HT_{1A}$ receptors in hippocampus was observed whereas the same risperidone regimen induced downregulation of NMDA receptor in hippocampus in adults. D2 receptor upregulation in NAc and striatum was observed in both adolescents and adults (Choi et al., 2009, 2010; Moran-Gates et al., 2007). The long-term effects on neurotransmitter receptors targeted by APDs remain largely unexplored.

The aim of the current study was to test the hypothesis that chronic adolescent exposure to risperidone, the atypical APD most commonly prescribed to adolescents (Hollingworth et al., 2013; Olfson et al., 2012; Ronsley et al., 2013), can induce long-lasting changes in brain structure, function and neurochemistry when compared with the same exposure in adults. We subjected the animals to chronic 21-day risperidone treatment either as adolescents (PND36-PND56) or adults (PND80-PND100). Risperidone-induced changes in neural function were assessed for alterations in (1) behaviour using the suppression of the CAR (2) brain structure using *in vivo* magnetic resonance imaging (MRI) and (3) neurochemistry in the NAc using real-time polymerase chain reaction (PCR). We chose to examine the behavioural changes with the CAR given that this behavioural paradigm can detect the effects of risperidone on integrated brain function with predictive validity, construct validity and reliability (Wadenberg, 2010; Wadenberg and Hicks, 1999). In addition to avoidance and chamber crossings that are usually reported with CAR paradigm in the literature, we examined risperidone-induced escape failure responses in adolescents and adults and the individual contributions of these behaviours to avoidance suppression. Changes in dopaminergic, serotonergic and GABA-ergic molecules in the NAc were examined since this brain region plays a critical role in APD-induced disruption of avoidance response (Wadenberg et al., 1990).

2. Materials and methods

2.1. Subjects

All procedures in this study were approved by the University of Queensland Animal Ethics Committee and followed the guidelines of the National Health and Medical Research Council of Australia. Male Sprague Dawley (SD) rats arrived at the animal facility as weaners on PND23. During the 7-day acclimatization period, they were housed in groups of eight in Macrolon cages (510 mm × 330 mm x 190 mm). After behavioural training from PND30 to PND34, the rats from the same age and drug groups were pair-housed in Macrolon cages (390 mm × 235 mm x 160 mm) with Sani chip bedding (Able Scientific) and wire lids in a temperature (21 ± 1 °C) and lighting (lights on at 6 a.m. and off at 6 p.m.) controlled room. All rats were given *ad libitum* access to food and water throughout the whole experiment. Behavioural training and testing were conducted during the light phase of the diurnal cycle. A separate cohort of sixteen 12–15-week old adult male SD rats was used to examine the effects of an acute single dose of risperidone on CAR.

2.2. Antipsychotic drugs (APDs)

Risperidone (Sigma Aldrich) was dissolved in 1% acetic acid in water and further diluted in sterile 0.9% normal saline (pH adjusted to 5.7–5.9), to make up to desired volume. Drug concentrations in the injectates were spectrophotometrically confirmed, using UV