Pharmacological Research 121 (2017) 1–13

Contents lists available at ScienceDirect

Pharmacological Research

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

Short- and long-term effects of risperidone on catalepsy sensitisation and acquisition of conditioned avoidance response: Adolescent vs adult rats

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ARTICLE INFO

Article history:
Received 11 April 2017
Accepted 11 April 2017
Available online 13 April 2017

Keywords:
Adolescent
Risperidone
Sensitisation
Conditioned avoidance response
Catalepsy

ABSTRACT

The effects of antipsychotic drugs (APDs) on the adolescent brain are poorly understood despite a dramatic increase in prescription of these drugs in adolescents over the past twenty years. Neuronal systems continue to be remodeled during adolescence. Therefore, when given in adolescence, antipsychotic drugs (APDs) have the potential to affect this remodeling. In this study we investigated the effects of chronic 22-day risperidone treatment (1.3 mg/kg/day) in both adolescent and adult rats. We examined short- and long-term changes in behaviour (catalepsy, locomotion and conditioned avoidance response (CAR)), and dopaminergic and serotonergic neurochemistry in the striatum and the nucleus accumbens. Here, we report that, both during chronic treatment and after a lengthy drug-free interval, risperidone induced a sensitised cataleptic response regardless of the age of exposure. Selectively in adolescents, risperidone-induced catalepsy was inversely correlated with striatal dopamine turnover immediately after chronic treatment. After a drug-free interval, a significant proportion of rats with prior adolescent risperidone treatment also failed to acquire CAR to a defined criterion. Our data provide evidence that the same chronic risperidone treatment regimen can induce contrasting short- and long-term neural outcomes in the adolescent and adult brains.

1. Introduction

Adolescence is a period spanning between 10 years and 19 years, and is a critical transition period from childhood to adulthood. During this period, several important changes occur, for example, growth spurt, rise in gonadal hormones and changes in behaviour [1]. More importantly, major maturation changes occur in several neural systems of the adolescent brain. Both longitudinal and cross-sectional studies have identified changes in cortical and subcortical brain structures in adolescent human brain (for example, see [2–7]). Concurrent with these maturation changes, onset of most psychiatric disorders, such as schizophrenia and mood disorders, which are often considered to be neurodevelopmental disorders, occurs during adolescence [8–10]. Correspondingly, prescription of antipsychotic drugs (APDs) to treat such disorders and off-label use for behavioural symptoms in adolescents and children has increased dramatically over the past twenty years [11–18]. However, effects of APDs on the adolescent brain are still poorly understood.

A period equivalent to human adolescence exists in rodents [19–21]. Therefore, adolescent neuropharmacological exposure can be modelled and examined in laboratory rodents under stringent conditions. Major maturational changes have been identified during rodent adolescence in terms of brain structures [22] and neurotransmitter systems, including dopaminergic and serotonergic systems (for example, see [23–26]), as well as gamma-aminobutyric acid (GABA)-ergic [27] and endocannabinoid systems [28,29]. Given these important neurotransmitter systems are targeted by APDs, the use of such agents in adolescence may produce long-term adverse effects.

Recent preclinical studies in rodents have started to identify the neurobiological consequences of adolescent APD treatment, in particular, long-lasting changes in behaviour and neurochemistry after a drug-free interval. For example, adolescent APD treatment has been reported to produce long-term behavioural

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http://dx.doi.org/10.1016/j.phrs.2017.04.013
1043-6618/

changes in adulthood such as deficits in fear conditioning and acquisition of a delayed non-match to sample task [30], increased amphetamine reward behaviour [31], altered locomotion and anxiety/depression-related phenotypes [32] and sensitised *suppression* of previously acquired conditioned avoidance response (CAR) [33–35]. Adolescent treatment with olanzapine has also been reported to induce long-lasting neurochemical changes such as altered dopaminergic neurotransmission in the nucleus accumbens (NAc) [31], reduction in GABA and glutamate in the NAc [36] and arrested maturation of D2 receptor-mediated corticostriatal electrophysiological responses in the NAc [37]. Moreover, long-term alterations in dopaminergic receptors and tyrosine hydroxylase expression could also occur in region- and sex-dependent manner following treatment with aripiprazole, olanzapine and risperidone during childhood and adolescent period [38]. Long-lasting alterations in protein levels of the prefrontal cortex which are involved in mitochondrial and cytoskeletal functions and cellular metabolism, have also been observed with adolescent risperidone treatment [39].

In our own previous study, we have shown adolescent APD exposure produces long-term alterations in behaviour compared with adult exposure [40]. We chose risperidone for detailed examination given that this is the most commonly prescribed APD to adolescents and children internationally [12,13,16]. We identified long-lasting neurobiological changes such as sensitised *suppression* of previously acquired CAR and downregulation of serotonergic $5HT_{2A}$ receptor expression in the NAc of risperidone-exposed adolescents but not adults. In addition, after 17 days of chronic risperidone, adolescent rats had less escape failures i.e. a failure to respond to both conditioned and unconditioned stimuli (CS and US), in our CAR paradigm compared to adults [40]. Escape failures induced by APDs in the CAR paradigm are not well-understood, but may reflect an increased cataleptic response which has been suggested as analogous to the well-described extrapyramidal side effects of APDs [41,42]. Therefore, we now wish to address cataleptic responses directly in animals chronically exposed to risperidone at either adolescence or adulthood.

Like certain psychomimetic agents, APDs have also been hypothesized to induce behavioural sensitisation, i.e. intensification of a behavioural effect during repeated treatment and/or following a drug-free interval [43,44]. Indeed, a number of studies have demonstrated APD-induced sensitization of the *suppression* of CAR and catalepsy. While APD-induced sensitisation in CAR *suppression* has been demonstrated in both adolescents (for example, see [33]) and adults (for example, see [45]), studies of catalepsy sensitisation have mostly utilized adult animals (for example, see [46,47]). To the best of our knowledge, only a single study has examined catalepsy sensitization response in adolescents and adults, reporting that adolescents developed lower level of catalepsy sensitisation than adults during repeated treatment with haloperidol, but not clozapine [48]. While the findings of this study support our hypothesis that risperidone could induce lower catalepsy sensitization in adolescents compared with adults, no study to date has examined chronic treatment with this APD in adolescents and adults with respect to catalepsy sensitisation. Therefore, our first aim was to assess whether chronic risperidone treatment could induce differential short- and long-term catalepsy sensitisation in adolescents and adults.

Another important question is whether adolescent APD treatment can induce long-term deficits in cognitive performance. A recent preclinical study suggested that adolescent olanzapine treatment could impair the rate of learning in a delayed non-matched to sample task in adulthood [30]. While these data are suggestive of an adolescent APD exposure impairing later learning, it is still unclear whether this deficit was selective to adolescent treatment given a lack of comparison age group. Here we aimed to assess long-term effects of risperidone on cognition by examining an animal's ability to *acquire* CAR after a lengthy drug-free interval following either adolescent or adult risperidone treatment. CAR is the gold-standard behavioural test used in the screening of novel compounds with APD potential [42,49]. In addition, the ability to acquire a CAR is frequently investigated in studies of fear and anxiety (for example, see [50,51]). Existing preclinical studies in rodents have investigated *acquisition* of the CAR in *adults* only *during* chronic treatment with risperidone [52,53]. No study to date has examined long-term effect of adolescent risperidone treatment on *first-time* CAR learning after a drug-free interval. Therefore, our second aim was to assess whether chronic risperidone treatment could induce differential abilities in adolescents and adults to acquire a CAR. In addition, in all experiments we examined monoamine levels and gene expression of dopaminergic receptors and dopamine-metabolizing enzymes in the striatum and the NAc that might contribute to observed behavioural changes. We chose to examine the levels of dopamine receptors in the striatum given their dynamic remodelling during adolescence [54,55] and given the reported roles of these receptors in catalepsy sensitisation [46].

2. Materials and methods

2.1. Subjects

Male Sprague Dawley (SD) rats were used. Rats arrived at the animal facility either on postnatal day (PND) 28 (for adolescent cohort) or PND 70 (for adult cohort). Rats from the same drug and age groups were pair-housed in Macrolon cages (39 cm x 23.5 cm x 16 cm) with Sani chip bedding (Able Scientific) and wire lids in a temperature ($21 \pm 1\,^{\circ}C$) and lighting (lights on at 6 am and off at 6 pm) controlled room. All rats were given ad libitum access to food and water throughout the whole experiment. Behavioural tests were conducted during the light phase of the diurnal cycle. 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.

2.2. Drug preparation

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. Control animals received vehicle solution (VEH) (1% acetic acid diluted with 0.9% normal saline at pH 5.7–5.9). Both risperidone (1.3 mg/kg/day) and vehicle were administered to the rats through once-daily intraperitoneal (IP) injection (1 ml/kg) for 22 days (between 12:00 and 3:30 pm). Rats were weighed daily approximately 30–60 min before drug administration. The risperidone dose chosen has been reported to achieve clinically relevant dopamine receptor occupancy of 60–80% [56,57] and we have shown this dose to induce robust changes in behaviour and neurochemistry in our previous study [40].

2.3. Experiment 1: examination of risperidone-induced short-term cataleptic and neurochemical responses

2.3.1. Experimental design

Rats were treated with risperidone or vehicle IP for 22 continuous days either as adolescents (PND35-PND56) or as adults (PND80-PND101) (Fig. 1, n = 12 per drug for a given age). At Days 1, 3, 5, 7, 10 and 17 of chronic treatment, rats were examined for cataleptic response (see below) using a horizontal bar test. Immediately after this, rats were placed in the locomotor chambers for