Psychiatry Research xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Psychiatry Research

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

# The effects of antipsychotics on the density of cannabinoid receptors in selected brain regions of male and female adolescent juvenile rats

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ARTICLE INFO

*Keywords:*
Aripiprazole
Risperidone
Olanzapine
Cannabinoid receptors
Adolescent rat

ABSTRACT

Antipsychotic drugs have been increasingly prescribed to children and adolescents for treating various mental disorders, such as childhood-onset schizophrenia. The abnormality of endocannabinoid system is involved in the pathophysiology of these disorders in juveniles. This study investigated the effect of antipsychotics on the cannabinoid (CB) receptors in the brain of both male and female juvenile rats. The postnatal rats (PD23 ± 1) were administered aripiprazole (1 mg/kg), olanzapine (1 mg/kg), risperidone (0.3 mg/kg) or vehicle (control) for 3 weeks. Quantitative autoradiography was used to investigate the binding densities of [$^3$H]CP-55940 (an agonist for CB1R and CB2R) and [$^3$H]SR141716A (a selective CB1R antagonist) in the rat brains. Risperidone significantly upregulated the [$^3$H]CP55940 and [$^3$H]SR141716A bindings in the prefrontal cortex (PFC), nucleus accumbens core (NAcC), nucleus accumbens shell (NAcS), cingulate cortex (Cg), and the caudate putamen (CPu) in male rats. Moreover, aripiprazole significantly elevated the [$^3$H]SR141716A binding in the Cg and NAcS of female rats. Furthermore, there is an overall higher [$^3$H]SR141716A binding level in the brain of female rats than male rats. Therefore, treatment with aripiprazole, olanzapine and risperidone could induce differential and gender specific effects on the binding density of cannabinoid receptors in the selected brain regions of childhood/adolescent rats.

## 1. Introduction

In recent years, prescriptions of atypical antipsychotic drugs (APDs) (mostly off-label use, particularly olanzapine, risperidone and aripiprazole), in children and adolescents have been dramatically increased for treating various mental disorders, such as childhood-onset schizophrenia, bipolar disorder, autism, attention deficit hyperactivity disorder, and Tourette's disorder (Ronsley et al., 2013). These APDs target multiple neurotransmission receptors, particularly dopamine D2 and serotonin (5-HT)2 receptors to achieve their therapeutic action (Ginovart and Kapur, 2012; Meltzer and Massey, 2011). Olanzapine and risperidone are antagonists at these neurotransmission receptors (Correll, 2010), while aripiprazole is a partial agonist of the dopamine D2 and 5-HT1A receptors, a partial antagonist of the 5-HT2A receptor, and is sometimes defined as a D2 receptor (D2R) functional selective drug (Di Sciascio and Riva, 2015; Levoyer et al., 2007; Mailman, 2007; Mailman and Murthy, 2010). It is important that those receptors also play crucial roles in neurodevelopment and almost all of the core brain functions (Ginovart and Kapur, 2012; Meltzer and Massey, 2011).

Childhood-adolescence is a critical period of neural development, with sculpting of the neuronal pruning, apoptosis and myelination (Schneider, 2008). Substances, such as APD treatment, during this crucial neurodevelopmental period may impact brain maturation and plasticity in brain neurotransmission (Ronsley et al., 2013; Schneider, 2008). Cannabinoid receptor mediates physiological and behavioural effects of natural and synthetic cannabinoids (Herkenham et al., 1991). It has been shown that developmental exposure to cannabinoids may induce subtle and long-lasting neuro-functional alterations (Trezza and Vanderschuren, 2008). Moreover, it has been indicated that the endocannabinoid CB1 receptors (CB1R) and their endogenous ligands are expressed in the early brain development period in rodents (Vitalis et al., 2008). The CB1R are widely distributed in the cerebral cortical brain regions of both humans and rats, including the prefrontal cortex (PFC), cingulate cortex (Cg), caudate putamen (CPu), and nucleus accumbens (NAc) (Breivogel and Childers, 1998; Derbenev et al., 2004). These brain regions are associated with the pathophysiology of

*Abbreviation:* 5-HT, serotonin; ADHD, attention deficit hyperactivity disorder; ANOVA, analysis of variance; APDs, antipsychotic drugs; BSA, bovine serum albumin; cAMP, cyclic adenosine monophosphate; Cg, cingulate cortex; CPu, caudate putamen; CB1R, cannabinoids 1 receptor; CB2R, cannabinoids 2 receptor; D2R, D2 receptor; NAc, nucleus accumbens; NAcC, nucleus accumbens core; NAcS, nucleus accumbens shell; PFC, prefrontal cortex; PD, postnatal day; RT, room temperature; SEM, standard error of mean

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https://doi.org/10.1016/j.psychres.2018.03.030
Received 30 August 2017; Received in revised form 5 January 2018; Accepted 8 March 2018

childhood schizophrenia, bipolar disorder, autism, and attention deficit hyperactivity disorder (ADHD) (Almeida et al., 2014; Zavitsanou et al., 2004), as well as the action of APDs (Cheng et al., 2008), but not involved directly in body weight regulation (Theisen et al., 2007). The elevation of CB1R binding was observed in the prefrontal area of brains from schizophrenic and other mental disorder patients (Ceccarini, 2014; Dalton et al., 2011; Jenko et al., 2012) Therefore, the endocannabinoid system plays a vital role in brain development and the pathology of various mental disorders (Renard et al., 2014), and contributes to the efficacy of APDs (Berk et al., 1999; Sundram et al., 2005).

Except for the endocannabinoid CB1R, the brain regions, including the PFC, Cg, CPu, and NAc also contain high levels of dopamine and 5-HT receptors; in these brain regions there are interactions between the monoamine neurotransmitters and the endocannabinoid system (Hermann et al., 2002; Wiley et al., 2008). The dopamine and 5-HT receptors are located in the mesocortical, mesolimbic and nigrostriatal pathways, which contribute to the effects of APDs on the function of cognition, affect and motor, respectively (Glass et al., 1997; Wiley et al., 2008). A number of studies have suggested the interaction between cannabinoid receptors and dopamine/5-HT receptors. As a selective CB1R antagonist, SR141716A upregulated the expression of dopamine, 5-HT, and noradrenaline in the PFC of rats (Tzavara et al., 2003). In the primary cell culture of striatal neurons, both the CB1R agonist HU210 and the D2R agonist quinpirole inhibited forskolin-stimulated cyclic adenosine monophosphate (cAMP) accumulation when applied separately, while HU210 and quinpirole in combination augmented cAMP accumulation, which was blocked by the CB1R antagonist SR141716A or the D2R antagonist sulphide (Glass et al., 1997). One previous study in the rat brain showed that the D2R level could be reduced by SR141716A through the CB1R in the extrapyramidal system (Alonso et al., 1999). On the other hand, it was reported that neurological and psychiatric disorders are often associated with long-term impairment of serotonergic and endocannabinoid control of synaptic plastic in the PFC (Ferreira et al., 2012). This is partially attributed to the highly overlapping distribution pattern of the cannabinoid CB1R and 5-HT receptors in the brain (Hermann et al., 2002). The previous study from our group reported that early treatment with various APDs had different effects on the 5-HT and dopamine receptors with some gender-dependent changes (Lian et al., 2016). It has been suggested that APDs increased CB1R availability in schizophrenia patients (Ranganathan et al., 2016). Therefore, APDs may regulate cannabinoid receptors through dopamine or serotonin neurotransmission.

It is worth noting that the onset of schizophrenia and bipolar disorder often occurs during late adolescence; therefore, evaluation of APD interaction with the endocannabinoid system in adolescents is indispensable (Wiley et al., 2008). Thus, to determine the effects of the APDs olanzapine, risperidone and aripiprazole on the endogenous cannabinoid system in the adolescent rat brain, the binding density of the cannabinoid CBR in the PFC, CPu, and NAc of adolescent rats was measured after 3 weeks' APD treatment.

## 2. Method

### 2.1. Animals, diet and experimental procedures

Timed pregnant Sprague Dawley rats (at gestation day 16) were obtained from the Animal Resources Centre (Perth, WA, Australia). They were housed in individual cages and allowed *ad-libitum* access to standard laboratory chow diet (3.9 kcal/g: 10% fat, 74% carbohydrate, 16% protein) and water under a light (07:00 to 19:00) and dark (19:00 to 7:00) cycle and temperature control (22 °C) throughout the experiment (Deng et al., 2012; Lian et al., 2014). Day of birth was recognised as postnatal day (PD) 0. Pups were sexed on PD14, and 24 male and female rats were weaned on PD21 and housed in individual cages.

Before the treatment procedures, rats were trained for self-administration of the drug by feeding them using 0.3 g cookie dough (including 30.9% cornstarch, 30.9% sucrose, 6.3% gelatine, 15.5% casein, 6.4% fibre, 8.4% minerals and 1.6% vitamins) without drug twice a day during PD18-21. There were four treatment groups: (1) Aripiprazole (1 mg/kg, 3 times/day, Otsuka, Japan; n = 6), (2) Olanzapine (1 mg/kg,3 times/day, Eli Lilly, USA; n = 6), (3) Risperidone (0.3 mg/kg, 3 times/day, Janssen, USA; n = 6) or (4) Vehicle (control; n = 6) for 3 weeks (a period corresponding to the childhood-adolescence period in humans) (Andersen, 2003). Drugs were prepared in advance by mixing with cookie dough pellets and droplets of water, and were administered 3 times per day (8 ± 1 h intervals) orally for 3 weeks (Lian et al., 2014). The rats in the control group received an equivalent pellet without drug. Rats were observed throughout the experiment to ensure all cookie dough pellets were consumed. This study was approved by the Animal Ethics Committee, University of Wollongong, Australia (AE12/20); and all the procedures complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).

### 2.2. Histological procedures

Forty eight hours after the last drug treatment, the rats were sacrificed, their brain tissue was removed and frozen in liquid nitrogen, and then stored at −80 °C until analysis. Brains were coronally sectioned at −18 °C into 14 µm using a cryostat (Leica CM1850, Leica Microsystem, Germany). Sections were thaw-mounted onto Polysine™ Microscope Slides (Menzel GmbH & Co. KG, Braunchweig, Germany) and stored at −20 °C. A set of sections from each animal was stained with 0.5% cresyl violet solution (Nissl staining) and used to confirm anatomical structures.

### 2.3. [$^3$H]CP-55940 binding

The cannabinoid receptor binding was performed using [$^3$H]CP-55940 (an agonist to CB1R and CB2R) as previously described (Deng et al., 2007). Briefly, brain sections containing the PFC, Cg, NAc and CPu were thawed at room temperature (RT), and after 30 min of pre-incubation in 50 mM Tris buffer (pH 7.4) containing 5% bovine serum albumin (BSA), they were incubated with 10 nM[$^3$H]CP-55940 (specific activity: 67 Ci/mmol; ki: 1.07 nM; PerkinElmer, USA) in 50 mM Tris buffer containing 5% BSA for 2 h at RT to determine total binding. Non-specific binding was determined by incubating the next sequential sections with 10 nM [$^3$H]CP-55940 incubation buffer, with the addition of 10 µM CP-55940 (Sigma Pharmaceuticals, Australia). Slides were washed for 5 min in ice-cold buffer, dipped in ice-cold distilled water, and then dried under a stream of cool air to remove excess buffer salts (Weston-Green et al., 2008).

### 2.4. [$^3$H]SR141716A binding

In brief, binding of [$^3$H]SR141716A (a CB1R selective antagonist, specific activity: 20.8 Ci/mmol, PerkinElmer, USA) using [$^3$H] SR141716A was performed based on procedures previously described (Deng et al., 2007). In brief, sections were pre-incubated in 50 nM Tris buffer including 5%BSA (pH 7.4) for 15 min at RT. Sections were then incubated for 1 h at RT in the same buffer containing 1.5 nM [$^3$H] SR141716A (specific activity: 67 Ci/mmol; ki: 2.04 nM; Amersham, UK) and 0.1% bovine serum albumin (BSA) for the total binding. Non-specific binding was determined with the addition of 100 µM HU210. After incubation, the sections were washed in ice-cold buffer containing 0.1% BSA (2 × 30 min), dipped in distilled water and air dried (Deng et al., 2007).

### 2.5. Autoradiography and quantification of cannabinoid receptor binding

All of the receptor binding slides were exposed to Kodak BioMax MR