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Early antipsychotic exposure affects serotonin and dopamine receptor binding density differently in selected brain loci of male and female juvenile rats

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ABSTRACT

Background: Antipsychotic drugs (APDs) were developed to treat schizophrenia in adults; however they have been increasingly prescribed (mostly "off-label") for children and adolescents. This study aimed to investigate the effects of aripiprazole, olanzapine and risperidone on the binding of serotonin and dopamine receptors in juvenile rat brain regions that are involved in antipsychotic efficacy.
Methods: Male and female rats were treated orally with aripiprazole (1 mg/kg), olanzapine (1 mg/kg), risperidone (0.3 mg/kg) or vehicle 3 times/day starting from postnatal day 23 (±1 day) for 20 days. Quantitative autoradiography was performed to examine the receptor binding densities.
Results: Olanzapine significantly decreased 5-HT_{2A} (5-HT_{2A}R) and 5-HT_{2C} receptor (5-HT_{2C}R) binding in the prefrontal cortex (PFC), cingulate cortex (Cg) and nucleus accumbens (NAc) of both male and female rats. In the caudate putamen (CPu), olanzapine attenuated 5-HT_{2A}R binding in both genders, and reduced 5-HT_{2C}R binding in male rats. Olanzapine increased D_2 receptor (D_2R) binding in the NAcS of male rats, but decreased it in females. Olanzapine increased D_1 receptor (D_1R) binding in the Cg, while aripiprazole decreased D_1R binding in the PFC of males. Aripiprazole significantly reduced 5-HT_{2A}R binding in the male PFC. Risperidone decreased 5-HT_{2A}R binding in the PFC of female rats, while attenuating D_1R binding in the PFC and Cg of males. However, APDs have no effects on the binding of serotonin and dopamine transporters.
Conclusion: This study revealed that aripiprazole, olanzapine and risperidone affected 5-HT_{2A}R, 5-HT_{2C}R, 5-HTT, D_1R and D_2R bindings differently in the brains of juvenile male and female rats.

Introduction

Over the past decade, there has been a sharp worldwide increase in prescription of antipsychotic drugs (APDs) (mostly off-label use), particularly atypical APDs olanzapine, risperidone and aripiprazole, in children and adolescents. These are prescribed to control various mental disorders including childhood-onset schizophrenia, bipolar disorder, autism, ADHD, Tourette's disorder, and anxiety disorder [1], despite a paucity of systematic investigations of their efficacy and side-effects [2]. These atypical APDs target multiple neurotransmission receptors, particularly dopamine and serotonin (5-HT) receptors for their therapeutic actions [3]. On the other hand, both dopaminergic and 5-HTergic neurotransmissions play a crucial role in neurodevelopment and almost all of the core brain functions [4]. However, limited studies have systematically investigated the effects of early exposure to these atypical APDs on these neurotransmissions during the childhood-adolescent period. The available evidence shows that olanzapine and risperidone have different effects on dopamine and 5-HT receptor binding in male juvenile rats compared to adult animals [5]. In view of the fact that there are gender differences in both brain development and antipsychotic effects in adults [6], it is very important to investigate whether there is gender difference in the effects of APDs on dopamine and 5-HT neurotransmission; to date, no study has been published in female juvenile animals. It is

Abbreviations: 5-HT_{2A}R, serotonin 5-HT_{2A} receptor; 5-HT_{2C}R, serotonin 5-HT_{2C} receptor; 5-HTT, serotonin 5-HT transporter; ANOVA, analysis of variance; APDs, antipsychotic drugs; Cg, cingulate cortex; CPu, caudate putamen; D_1R, dopamine D_1 receptor; D_2R, dopamine D_2 receptor; DFC, dorsolateral frontal cerebral cortex; DAT, dopamine transporter; NAc, nucleus accumbens; NAcC, nucleus accumbens core; NAcS, nucleus accumbens shell; PFC, prefrontal cortex; PD, postnatal day.

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also worth noting that APDs were delivered through single daily injections in previous studies, which does not closely mimic clinical treatment conditions due to a shorter half-life of APDs in rodents than in humans [7]. Therefore, this study investigated the effects of oral treatment (3 times per day) of aripiprazole, olanzapine and risperidone at a clinical equivalent dosage (a better mimicking of the clinical treatment paradigm) in the childhood/adolescence period on the dopaminergic and 5-HTergic systems in both male and female juvenile rats.

Materials and methods

Animals and drug administration

Fourteen timed pregnant Sprague-Dawley rats were obtained at gestation day 14 from the Animal Resource Centre (Perth, WA, Australia). They were housed individually at 22 °C, on a 12-h light–dark cycle (lights on: 07:00 h and light off: 19:00), and allowed *ad libitum* access to water and standard laboratory chow diet through the whole experiment [8]. Forty-eight (including 24 males and 24 females) rats born from these pregnant rats were used in this study. Day of birth was considered postnatal day (PN) 0. Pups were weaned at PN21 and were housed individually. After weaning, young rats (n = 6/group) were randomly assigned to one of the following treatments starting from PN23 (±1 day) for 20 days (a period corresponding to the childhood-adolescence period in humans [9]: 1.0 mg/kg aripiprazole (Bristol-Myers Squibb, New York, USA), 1.0 mg/kg olanzapine (Eli Lilly, Indianapolis, IN, USA), 0.3 mg/kg risperidone (Apotex, Macquarie Park, NSW, Australia), or vehicle (control). The pellets with drugs were made prior to administration by mixing droplets of water with cookie dough powder (containing 30.9% cornstarch, 30.9% sucrose, 6.3% gelatine, 15.5% casein, 6.4% fibre, 8.4% minerals, and 1.6% vitamins) [8,10]. Controls received an equivalent pellet without the drug. Animals were observed during treatment administration to ensure complete consumption of each pellet. Prior to the drug treatment, animals underwent a teaching period to self-administer a sweet cookie dough pellet from PN17 for 6 day.

The drug doses used in this study are translated from human doses based on the formula in the FDA guideline for clinical trials [11,12], which are within the recommended dosage ranges for the psychiatric treatment of children and adolescents [13,14]. In consideration of the shorter half-life of these antipsychotic drugs in rats [7,15,16], the rats were administered antipsychotic drugs three times/day (8 ± 1 h interval) to ensure consistently high concentrations to better mirror the human scenario of oral administration once per day [8]. All experimental procedures were approved by the Animal Ethics Committee, University of Wollongong, and complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).

Histological procedures

After two days drug wash-out period following the last drug treatment, all rats were sacrificed using carbon dioxide asphyxiation, and their brains were removed and frozen in liquid nitrogen immediately followed by storage in a −80 °C freezer until sectioning. Brains were cut at −18 °C into 14 μm coronal sections using a Leica CM1850 cryostat (Leica Microsystems, Germany). The corresponding brain regions were obtained based on a standard rat brain atlas [17] (Fig. 1). Brain sections were thaw-mounted onto Polysine™ Microscope Slides (Menzel GmbH & Co. KG, Braunschweig, 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 for confirmation of anatomical structures.

Serotonin receptor and transporter binding

The methods for the serotonin 5-HT_{2A} receptor (5-HT_{2A}R), 5-HT_{2C} (5-HT_{2C}R) and 5-HT transporter (5-HTT) binding autoradiography have been reported previously [18,19]. In brief, brain sections containing the PFC, Cg, NAc and CPu were thawed at room temperature (RT) and then were pre-incubated for 15 min in 170 mM Tris buffer (pH 7.4, for 5-HT_{2A}R and 5-HT_{2C}R bindings) or 50 mM Tris buffer with 120 mM NaCl and 5 mM KCl (for 5-HTT binding) (pH 7.4) at RT. They were then incubated for 2 h at RT with 2 nM [3H]ketanserin (for 5-HT_{2A}R binding; Specific activity: 67 Ci/mmol; PerkinElmer, Waltham, MA, USA), 5 nM [3H]mesulergine (for 5-HT_{2C}R binding, 84.5 Ci/mmol, PerkinElmer), and 0.6 nM [3H]paroxetine (for 5-HTT binding; 20.8 Ci/mmol, PerkinElmer), respectively to determine total binding. Non-specific binding was detected with the presence of 2 μM spiperone (Sigma Pharmaceuticals, Australia) for [3H]ketanserin binding, or 100 nM spiperone and 10 μM mianserin for [^{3}H]mesulergine binding, or 10 μM fluoxetine for [^{3}H]paroxetine binding, respectively. Sections were then washed four times for 5 mins (for 5-HT_{2A}R binding), or twice for 10 mins (for 5-HT_{2C}R and 5-HTT bindings) in ice-cold buffer, dipped in distilled water and air-dried.

Dopamine receptor and transporter binding

The procedures for dopamine D_1 receptor (D_1R), D_2 receptor (D_2R) and transporter (DAT) binding autoradiography were followed previous reports [20,21]. In brief, for D_1R binding the thawed slides were pre-incubated at RT in 50 mM Tris-HCl buffer together with 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$ (pH 7.4) for 20 mins. They were then briefly air-dried and incubated for 1.5 h at RT with 4 nM [^{3}H]SCH23390 (specific activity, 84 Ci/mmol) and 30 nM spiperone to prevent non-specific binding to the D_2R. Non-specific binding was determined by the addition of 10 μM (+)-butaclamol to adjacent sections.

For D_2R binding, the slides were pre-incubated at RT in 50 mM Tris-HCl buffer together with 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and 0.001% ascorbic acid (pH 7.4) for 20 mins; followed by 1 h of incubation in 5 nM [^{3}H]raclopride (specific activity: 60.1 Ci/mmol; PerkinElmer, USA). Non-specific binding was determined by 10 μM butaclamol and 5 nM [^{3}H]raclopride using the same buffer.

In terms of DAT binding, sections were pre-incubated in 50 mM Tris-HCl containing 120 mM NaCl and 0.1% bovine serum albumin (BSA; pH7.4) for 20 mins at 4 °C and then incubated for 2 h in the same buffer containing 15 nM [^{3}H]WIN35,428 (specific activity 85.6 Ci/mmol, Perkin-Elmer, USA). Nonspecific binding was determined in the presence of 50 μM benztropine.

Quantification and statistical analysis

After the various binding experiments, all the slides were exposed to Kodak BioMax MR film for 2–3 months with the standard autoradiographic bar ([3H]microscales from Amersham) in X-ray film cassettes. The films were developed at room temperature in Kodak X-ray developer and fixer. Autoradiographic images were quantitatively analysed using a Multi Analyst program (Bio-Rad, USA), then optical density measurement was converted into fmoles [^{3}H] ligand per mg TE (tissue equivalent) by comparing to the standard. Specific binding was calculated by subtracting non-specific binding from total binding. A set of sections from all cases was stained with cresyl violet. Specific brain regions in this project were identified by reference to the Nissl-stained sections and a standard rat brain atlas [17]. Statistical analysis was performed using SPSS (IBM version 19.0, IBM, USA). The Kolmogorov-Smirnov test was used to examine the