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Research report

Two models for weight gain and hyperphagia as side effects of atypical antipsychotics in male rats: Validation with olanzapine and ziprasidone

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ABSTRACT

Body weight gain is one of the most serious side effects associated with clinical use of antipsychotics. However, the mechanisms by which antipsychotics induce body weight gain are unknown, and no reliable animal models of antipsychotics-induced weight gain have been established. The present studies were designed to establish male rat models of weight gain induced by chronic and acute treatment with antipsychotics. Six-week chronic treatment with olanzapine (5, 7.5, and 10 mg/kg/day) in male Sprague-Dawley rats fed a daily diet resembling a human macronutrient diet, significantly increased body weight gain and weight of fatty tissues. In contrast, ziprasidone (1.25, 2.5, and 5 mg/kg/day) administration caused no observable adverse effects. We then investigated feeding behavior with acute antipsychotic treatment in male rats using an automated food measurement apparatus. Rats were allowed restricted access to normal laboratory chow (4 h/day). With acute olanzapine (0.5, 1, and 2 mg/kg, i.p.) treatment in the light phase, food intake volume and duration were significantly increased, while treatment with ziprasidone (0.3, 1, and 3 mg/kg, i.p.) did not increase food intake volume or meal time duration. Findings from the present studies showed that chronic treatment with olanzapine in male rats induced body weight gain, and acute injection induced hyperphagia, suggesting that hyperphagia may be involved in the weight gain and obesity-inducing properties of chronically administered olanzapine. These animal models may provide useful experimental platforms for analysis of the mechanism of hyperphagia and evaluating the potential risk of novel antipsychotics to induce weight gain in humans.

1. Introduction

Atypical antipsychotics have proven useful treatment options for schizophrenic patients, exhibiting lower liability for extrapyramidal symptoms than typical antipsychotics. However, most of these atypical antipsychotics are known to induce substantial body weight gain in patients [39,44]. This antipsychotic-induced body weight gain often leads to discontinuation of treatment, resulting in relapse of psychotic symptoms [29,50]. Among conventional antipsychotics, olanzapine and clozapine are reported to be the most serious weight gain-inducing drugs administered to schizophrenia patients, as revealed by meta-analysis [3,31]. In contrast to olanzapine and clozapine, drugs such as quetiapine, risperidone and aripiprazole have relatively moderate liability, and ziprasidone is known as a "weight neutral drug". On meta-analysis of 10-week body weight gain in humans, olanzapine and ziprasidone induced body weight gains of 4.15 and 0.04 kg, respectively.

A considerable number of pre-clinical studies have been conducted to reproduce these metabolic side effects, particularly antipsychotics-induced hyperphagia and weight-gain occasionally observed in clinical settings. In experimental animals, many reports have demonstrated that antipsychotics can more easily induce body weight gain and hyperphagia in female rodents than in males [1,11–13]. However, no pre-clinical model with both robust reproducibility and predictive validity has yet been established. For instance, although olanzapine has a much shorter plasma half life in rats than in humans (1.4 h vs. 30 h) [8,11], treatment only once or twice per day with olanzapine induces increases in body weight and food intake and adiposity within 1 to 3 weeks of commencement of chronic treatment in female rats [4,12,17,19,20,24,25]. In contrast, male rats show no such increase in body weight or food intake under similar experimental conditions [1,6,13,41], even with chronic pump infusion [11]. This gender difference is unlikely to be due to pharmacokinetic differences in acute and chronic

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injection [11,41]. In addition, antipsychotics are known to occasionally induce "false positives" (ziprasidone or aripiprazole induced weight gain) [24,25] in female rats. Female rodents appear to be more sensitive than humans with regard to susceptibility to hyperphagia and weight gain, and the predictive validity of a model using female rodents is thought to be limited [24,25]. One possible reason for these observed inconsistencies is the antipsychotics' effects on prolactin. Some studies suggest that hyperprolactinemia through dopamine D_2 receptor antagonism may be involved in the hyperphagia [5]. Given that lactation is a heavier burden in female rodents than in humans, hyperprolactinemia has been hypothesized to induce hyperphagia in female rodents [23]. However, several studies have failed to demonstrate the relationship between hyperphagia (or body weight gain) and hyperprolactinemia induced by antipsychotics in female rats [12,24,25]. As such, the low predictive validity of the female rodent model remains inexplicable.

However, olanzapine has been found incapable of inducing hyperphagia or weight gain even on repeated administration in male rodents [13], which naturally do not exhibit hyperphagia due to lactation induced by prolactin. Investigations involving male animals are therefore needed to thoroughly analyze the mechanisms underlying weight gain and develop new drugs with low liability for weight gain. To our knowledge, only one group has reported on body weight gain in chronic olanzapine-treated male rats [36], with results showing that olanzapine induced both hyperphagia and weight gain, but not by ziprasidone. Although these previous findings suggest that the male rat model may have better predictive validity than females, the effective dosages of olanzapine in these reports are inconsistent [37], possibly due to low robustness of olanzapine-induced effects on hyperphagia and body weight gain in male rats under the experimental conditions.

The present series of studies was designed to establish a reliable model of body weight gain and appetite in male rats which is expected to have better predictive validity than models previously reported. Our first study was dedicated to investigate the consequences of chronic treatment with atypical antipsychotics on body weight, food intake, and fat weight in male rats fed a special diet [36]. In addition, as olanzapine induced hyperphagia and weight gain, we also measured the plasma concentration of olanzapine in attempt to clarify if the effects are related to those in humans. To validate the model, two atypical antipsychotics olanzapine and ziprasidone, which are respectively reported to be a positive and a negative control, were selected.

In addition, to achieve more robust detection of olanzapine-induced hyperphagia in male rats, we developed a novel methodology which uses an automatically-controlled food intake measurement apparatus and evaluated the effects of acute administration of olanzapine and ziprasidone on food intake. Meal time duration was restricted to four hours per day, and the effects of drugs were examined after acclimatization to the meal schedule. Given that olanzapine has a shorter plasma half-life in rats than in humans, we used rats that had been acclimatized to short food intake times in order to evaluate the effects while drug plasma concentrations were sufficiently high. Dosages of olanzapine and ziprasidone were chosen based on data regarding *in vivo* antipsychotic-like effects. Attenuation of amphetamine-induced hyperlocomotion in male rats was used to assess antipsychotic-like efficacy.

2. Materials and methods

2.1. Drugs

Olanzapine (brand name: Zyprexa) and ziprasidone (brand name: Geodon) were purchased from Eli Lilly (Indianapolis, IN, USA) and Pfizer, Inc. (New York City, NY, USA), respectively, and extracted at Astellas Pharma Inc. (Tsukuba, Japan). D-Amphetamine sulfate was synthesized at Yamanouchi Pharma Inc. (now Astellas Pharma Inc., Tsukuba, Japan) under the permission of the Ministry of Health, Labor and Welfare in Japan.

2.2. Animals

Male Sprague-Dawley (SD) rats were purchased from Japan SLC, Inc. (Shizuoka, Japan) at age 6 weeks and used at age 7–8 weeks. Rats were housed in a temperature- and humidity-controlled colony room ($23 \pm 3\,^{\circ}C$ and $55 \pm 15\%$, respectively) under a 12-h light/12-h dark cycle with *ad libitum* access to water and laboratory rodent chow (CE-2, CLEA Japan, Inc., Tokyo, Japan). Rats were maintained three per cage during acclimation. Procedures involving animals and their care were conducted in accordance with the institutional guidelines of Astellas Pharma Inc., which are in compliance with international laws and policies (International Guiding Principles for Biomedical Research Involving Animals, developed by the Council for International Organization of Medical Science). All experiments were also conducted in accordance with the Astellas Pharma Inc. guidelines for the care and use of animals and under approved protocols from the Institutional Animal Care and Use Committee of Astellas Pharma Inc.

2.3. Amphetamine-induced hyperlocomotion test

Male SD rats were orally (p.o.) injected with vehicle or drugs 30 min before, or intraperitoneally (i.p.) injected just before placement in the experimental open field (L × W × H: 500 mm × 500 mm × 300 mm). Thirty minutes after placement, rats were injected with d-amphetamine sulfate (1 mg/kg, subcutaneous [s.c.]), and locomotor activity was immediately recorded for 60 min using a photobeam activity monitor and sensor unit (PAM-1 and BAT-1; Muromachi Kikai Co., Ltd., Tokyo, Japan). Horizontal movements were detected as activity counts, and data were analyzed using CompAct AMS ver. 3 (Muromachi Kikai Co., Ltd.). D-Amphetamine sulfate was dissolved in saline and injected at 1 mg/kg (s.c.). For i.p. injection, olanzapine (0.1–3 mg/kg) was dissolved with a drop of hydrochloric acid and diluted with distilled water. Ziprasidone (0.03–10 mg/kg, i.p.) was dissolved in distilled water alone. For oral injection, both compounds (1–10 mg/kg) were suspended in 0.5% methyl cellulose in distilled water. All injection volumes were 2 ml/kg. The test doses were determined based on the results of our preliminary experiments.

2.4. Effects of chronic administration of olanzapine and ziprasidone on body weight and fat weight

For chronic treatment of drugs, high carbohydrate-low protein powdered food was prepared according to the method proposed by Minet-Ringuet et al. [36]. Standard diet for rats consists of high-protein (35% of daily energy), high-lipid (50%), and low-carbohydrate food stuffs (15%); however, in the present study, we have used a diet that was low in protein (14%), high in carbohydrates (54%) and composed of 31% fat (P14C54L31) [33,36], which its protein/lipid/carbohydrate ratio was similar to human diet [49]. Specifically, the diet used in the present study consisted of 140 g/kg of whole milk protein, 538.1 g/kg of cornstarch, 87.6 g/kg of sucrose, and 137 g/kg of soybean oil. This diet was supplemented with mineral and vitamins according to the AIN-93 M requirements (mineral salts: 3.5 g/kg, vitamins: 10 g/kg, cellulose: 50 g/kg, and chlorine: 2.3 g/kg) [36,42]. Olanzapine and ziprasidone were weighed, pulverized with an agate mortar, and then mixed in with the powdered food. Olanzapine was mixed at 0.005%, 0.0075%, or 0.01% (vol) per gram weight, and ziprasidone was mixed at 0.00125%, 0.0025%, or 0.005% (vol) per gram weight. These values corresponded to 5, 7.5, and 10 mg/kg/day for olanzapine, and 1.25, 2.5, and 5 mg/kg/day for ziprasidone at initiation of experiments.

Since drugs were mixed at a constant ratio, dosages were gradually reduced with increasing animal body weight. For chronic treatment as a part of the diet, dosages of ziprasidone were determined based on the ED_{50} ratio of olanzapine to ziprasidone in amphetamine-induced hyperlocomotion test with oral injection of compounds (Table 1).

At least seven days before start of the experiment, rats were moved into individual bracket-type wire mesh cages (including an automatic washer belt under the cage; L × W × H: 39 cm × 45 cm × 22 cm; NATSUME SEISAKUSHO Co., Ltd.,

Table 1
ED_{50} values of amphetamine-induced hyperlocomotion test.

	ED_{50} (mg/kg)	
	i.p.	p.o.
Olanzapine	2.2 (1.7–3.0)	3.7 (3.1–4.4)
Ziprasidone	0.79 (0.5–1.3)	1.9 (1.5–2.3)

Olanzapine, ziprasidone, or vehicle was intraperitoneally (i.p.) administered 30 min before or orally (p.o.) administered 60 min before injection of d-amphetamine (1 mg/kg, subcutaneous). Locomotor activity counts were measured for 60 min after injection of saline or d-amphetamine. Parentheses indicate 95% confidence intervals of ED_{50} values. Each experiment was conducted with 3–6 doses, determined from the preliminary study. $n = 5$–8 rats per dose.