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Atypical antipsychotics such as olanzapine often induce excessive weight gain and type 2 diabetes. However, the mechanisms underlying these drug-induced metabolic perturbations remain poorly understood. Here, we used an experimental model that reproduces olanzapine-induced hyperphagia and obesity in female C57BL/6 mice. We found that olanzapine treatment acutely increased food intake, impaired glucose tolerance, and altered physical activity and energy expenditure in mice. Furthermore, olanzapine-induced hyperphagia and weight gain were blunted in mice lacking the serotonin 2C receptor (HTR2C). Finally, we showed that treatment with the HTR2C-specific agonist lorcaserin suppressed olanzapine-induced hyperphagia and weight gain. Lorcaserin treatment also improved glucose tolerance in olanzapine-fed mice. Collectively, our studies suggest that olanzapine exerts some of its untoward metabolic effects via antagonism of HTR2C.

Introduction

Atypical antipsychotics (AATPs) are second-generation antipsychotics that are currently used to treat a variety of psychiatric conditions, including schizophrenia, bipolar disorder, depression, and autism (1). Despite their documented efficacy and low risks for extrapyramidal symptoms, most AATPs have been linked to drug-induced metabolic side effects, including excessive weight gain, dyslipidemia, and type 2 diabetes (2). Notably, schizophrenic patients have a reduced life span, with obesity-related metabolic syndrome being the leading cause of death (2). Moreover, the incidence of diabetes among AATP users is 4 times higher than in age-, race-, and sex-matched controls (3). While morbid obesity and type 2 diabetes typically take years to unfold in the general population, these conditions manifest acutely (within months) following AATP treatment (4). The rapid disease onset suggests a distinct etiology underlying AATP-induced metabolic syndrome that remains poorly understood.

AATPs bind to multiple monoamine receptors in the brain (5). The beneficial psychotropic effects are thought to be mediated primarily by a combinatorial blockade of serotonin 2a and dopamine D2 receptors (6). However, the molecular mechanisms that underlie AATPs’ untoward metabolic effects remain largely unknown. Both genome-wide association and candidate studies in human patients have suggested the involvement of multiple genes, including those that encode the histamine, α-adrenergic, and serotonin (5-HT) receptors (7–9). Among them, Htr2c encodes the 5-HT 2C receptor, which acts in the brain to regulate food intake, body weight, and glucose metabolism (10, 11).

Blockade of HTR2C signaling in mice leads to hyperphagia and obesity (12) that resemble AATP-induced metabolic symptoms in humans. Many AATPs, including olanzapine, antagonize HTR2C (13), raising the possibility that Htr2c antagonism contributes to AATP-induced metabolic syndrome (14, 15). However, previous efforts to test this hypothesis using genetic models have been hindered by the difficulty of replicating AATP-induced metabolic perturbations in mice (16). Although it is one of the most commonly prescribed and effective AATPs, olanzapine causes the most weight gain and metabolic impairments in humans (17). To characterize its metabolic effects, we adopted an olanzapine-supplemented diet to reproduce human plasma olanzapine levels in female C57BL/6 mice (18). In the current study, we investigated the role of Htr2c in olanzapine-induced metabolic impairments in mice. We also assessed whether an HTR2C-specific agonist alleviates olanzapine’s untoward metabolic effects.

Results and Discussion

We found that chronic olanzapine exposure of female C57BL/6 mice resulted in excessive weight gain over a 6-week period (Figure 1A). Nuclear magnetic resonance (NMR) analysis revealed an increase in fat mass, but not lean mass, in olanzapine-fed mice (Figure 1B). In addition to causing obesity, chronic olanzapine treatment impaired glucose tolerance (Figure 1C). Moreover, fasting plasma insulin levels were significantly higher in olanzapine-fed mice (Figure 1D). To further characterize olanzapine’s acute effects on energy balance, we assessed another cohort of mice in a TSE Systems metabolic chamber system in which food intake, energy expenditure, and physical activity were monitored for a total of 6 days. Mice were fed a control diet (D09092903; Research Diets Inc., 45 kcal% fat, 35 kcal% carbohydrate, 15 kcal% protein) during acclimation and on the first 3 days in the metabolic cages. Mice were then switched to the olanzapine diet (50 mg olanzapine compounded into 1 kg of the control diet, Research Diets Inc.) for the next 3 days. Olanzapine is associated with food...
mice was similar to that in control mice during the same period, suggesting that hyperphagia is required for olanzapine-induced weight gain (Figure 1H).

The olanzapine-induced hyperphagia and weight gain allowed us to use genetically modified mice to investigate candidate genes and pathways that underlie these metabolic perturbations. To determine whether olanzapine acts on Htr2c to affect energy balance, we used mice lacking Htr2c (Htr2c-null mice, maintained on a C57BL/6 background). Notably, we found that olanzapine’s effect on weight gain was significantly blunted in Htr2c-null mice (Figure 2A). In contrast with WT mice, olanzapine-fed Htr2c-null mice had body weight and body composition comparable to those fed the control diet (Figure 2, A and B). Furthermore, olanzapine treatment did not significantly alter glucose tolerance or fasting plasma insulin levels in Htr2c-null mice (Figure 2, C and D). We next repeated the metabolic cage analysis in Htr2c-null mice and found that hyperphagia did not develop in Htr2c-null mice following acute olanzapine exposure (Figure 2E and Supplemental Figure 3A). In contrast, olanzapine’s effects on physical activity and energy expenditure persisted in these mice. Similarly to WT mice, Htr2c-null mice exhibited reduced activity (Figure 2F) and an increase in heat production (Figure 2G), O2 consumption, and CO2 production (Supplemental Figure 3, B–D) after the dietary switch. Collectively, our findings suggest that olanzapine’s effects on food intake and weight gain require Htr2c, whereas its effects on physical activity and energy expenditure likely involve additional receptors. Consistent with this observation, Chee and colleagues recently reported that melanin-concentrating hormone (MCH) is necessary for olanzapine suppression of locomotor activity (21).

Our finding that Htr2c antagonism contributes to olanzapine-induced hyperphagia and weight gain raises the possibility that agonists for HTR2C may be useful in alleviating these untoward effects. To this end, we fed female C57BL/6 mice the olanzapine

**Figure 1. Olanzapine treatment profoundly alters energy homeostasis in female C57BL/6 mice.** (A) Body weight. (B) Body composition. (C) GTT. (D) Plasma insulin levels. (E–G) Metabolic cage analysis (n = 6) of food intake (E), physical activity (F), and heat production (G). (H) Weight gain in ad libitum- and pair-fed mice. Results are shown as mean ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001 versus other genotypes assessed using either Student’s t test or 2-way ANOVA with Sidak’s multiple comparisons test. OLZ, olanzapine treated; Con, control.
diet for 6 weeks. These mice became obese compared with those fed the control diet (Figure 3B). We then treated mice with lorcaserin, an FDA-approved specific HTR2C agonist, or a vehicle for 7 days while still feeding the olanzapine diet (Figure 3A). We used an anorexigenic dose (10 mg/kg) that suppressed food intake in overnight-fasted mice (Supplemental Figure 4). We measured food intake and body weight daily during the treatment as well as 2 weeks after the last dose of lorcaserin or vehicle (Figure 3A). Notably, hyperphagia was evident in olanzapine-fed mice, but was attenuated by lorcaserin treatment (Figure 3C). Consistent with the reduction in food intake, we found that lorcaserin acutely suppressed weight gain in olanzapine-fed mice (Figure 3D). Importantly, we observed a significant improvement in glucose tolerance (day 8, Figure 3F). Nevertheless, the attenuation of weight gain and improvement in glucose tolerance did not persist after cessation of lorcaserin treatment (Figure 3, E and G, day 22).

In summary, our findings support the model that olanzapine acts on multiple receptors and pathways to alter different aspects of energy homeostasis. Notably, our data suggest that olanzapine-induced hyperphagia is the primary cause of weight gain in mice and that olanzapine’s effect on food intake is mediated by Htr2c. Furthermore, we demonstrate that olanzapine-induced hyperphagia is reduced by a HTR2C-specific agonist treatment in olanzapine-fed mice, accompanied by an improvement in glu-
cose homeostasis. Currently, there is no medication specifically targeting AATP-induced metabolic syndrome. Moreover, existing antiobesity and antidiabetic medications only provide limited relief (22, 23). Therefore, our findings suggest that available HTR2C-speciﬁc agonists, such as lorcaserin, may be useful in preventing some of the metabolic side effects associated with the use of olanzapine and other AATPs.

Methods
All experiments were repeated with at least 2 independent cohorts of mice.

Mice. All mice were housed in a temperature-controlled room with a 12-hour light/12-hour dark cycle (lights on at 06:00 am, lights off at 6:00 pm) in the animal facility of the UT Southwestern Medical Center. Mice were provided standard chow (no. 2016; Harlan Teklad) as well as water ad libitum unless otherwise noted. C57BL/6 mice were purchased from the UT Southwestern Rodent Breeding Core Facility. Htr2c-null mice were maintained on a C57BL/6 background. Htr2c-null mice were generated by inserting a transcriptional blocker cassette into the endogenous Htr2c allele. These mice are phenotypically identical to conventional Htr2c-null mice, as we previously described (11).

Body weight and composition. Body weight was monitored weekly between weaning (at 4 weeks) and up to 20 weeks of age. Body composition was assessed using the Bruker Minispec mq10 NMR analyzer.

Metabolic cage studies. These studies were conducted in the UT Southwestern Metabolic Phenotyping Core Facility. Data for food intake, energy expenditure, and physical activities were collected using a combined indirect calorimetry system (TSE Systems). Experimental animals were individually acclimated in the metabolic chambers for 5 days before data collection. During data collection, mice were initially maintained on the control diet for the ﬁrst 3 days and switched to the olanzapine diet for the next 3 days in the metabolic cages.

Glucose tolerance test, glucose, and plasma insulin levels. For glucose tolerance tests (GTTs), mice were fasted for 6 hours with water provided ad libitum from 9 am on the experimental day. Baseline glucose and insulin levels were collected 2 hours prior to GTT. During GTT, blood glucose levels were monitored at 15, 30, 60, 90, and 120 minutes after an i.p. dose of glucose (1 g/kg body weight). Blood glucose was analyzed using a Contour Glucometer (Bayer Pharma AG). Plasma insulin levels were measured using an ELISA kit designed for use in mice (Crystal Chem Inc.).

Feeding studies. For the refeeding experiment, mice were fasted for 18 hours (from 3 pm to 9 am) before being given an i.p. dose of saline or lorcaserin (MedChem Express, HY-153685, 5 or 10 mg/kg dissolved in saline). Thirty minutes after the injection, a chow pellet was given to singly housed mice. Food consumption was monitored at 30, 60, 120, and 240 minutes. For the pair-feeding experiment, 30 mice were individually housed and fed the control diet for a week. Food intake was monitored to calculate the average daily intake for each mouse. Sixteen mice that had similar amounts of daily intake (~2.9 g of the control diet) were used for the pair-feeding experiment. Mice were randomized into 2 groups with equal numbers and body weight. Mice in the control group continued to be fed the control diet, whereas those in the olanzapine group were fed the olanzapine diet for the next 7 days. In both groups, individual mice were fed the exact same amount of daily intake they consumed in the previous week. The other 14 mice were used as ad libitum controls. They were randomized into 2 groups and fed either the control diet or the olanzapine diet for 7 days. All mice remained individually housed during the pair-feeding period.

Statistics. Data were analyzed with either Student’s t test or 2-way repeated-measures ANOVA, as appropriate, followed by Sidak’s multiple comparisons test using Prism 7.0 (GraphPad). Statistical signiﬁcance was established at P < 0.05.

Study approval. All experimental procedures were approved by the IACUC at UT Southwestern Medical Center (animal protocol no. 2015-101099; principal investigator, C. Liu).

Author contributions
CCL, JKE, and CL designed the experiments. CCL, SCW, RW, CMC, NA, DM, and CL collected data. CCL, SCW, JKE, and CL analyzed the data. CCL, SL, JKE, and CL wrote the manuscript.

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