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Dysregulation of hypothalamic modulation in olanzapine treated male rats

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ABSTRACT

The mechanism of weight gain through application of olanzapine, a serotonin and dopamine receptor antagonist has not been fully understood. Weight gain and food intake are under the control of various neurohormones; POMC (proopiomelanocortin), CART (cocaine and amphetamine regulated transcript), AgRP (Agouti-related peptide) and NPY (neuropeptide Y) that are majorly synthesized and secreted from the arcuate nucleus (ARC) of hypothalamus. In this study, the alteration of the ARC neurohormone levels in rats were determined as one of the weight gain mechanisms. To understand the underlying mechanism of olanzapine-induced weight gain, the drug was orally administrated to healthy male Wistar rats for analysis of both the hypothalamic gene expression and peripheral levels of those candidate neuropeptides. In rats hypothalamic mRNA levels of *NPY*, *AgRP* and *POMC* decreased while *CART* levels did not show any alteration. Consistently, circulating levels of NPY, AgRP and α-MSH decreased significantly yet CART levels were also reduced. In conclusion, it may be presumed that the antagonistic effect of olanzapine on the ARC neurons might be the onset for a dysregulation of the neurohormones secretion which may cause weight gain during treatment.

1. Introduction

The treatment of psychosis, particularly schizophrenia, is accomplished by the use of antipsychotics, which are divided into two groups as typical and atypical (Meltzer, 2013). Atypical antipsychotics are known to have stronger effects on ameliorating the negative and positive symptoms of schizophrenia (Wagner et al., 2005). Atypical antipsychotics differ from typical antipsychotics in terms of their binding affinities to serotonergic and dopaminergic receptors which result in fewer side effects. They bind dopaminergic receptors, particularly D2 more loosely and are released more quickly. The quick release from D2 receptors results in reduced risk of developing tardive dyskinesia, involuntarily repetitive movements of the body commonly seen in the long term usage of antipsychotics (Seeman, 2002). While preventing such side effects, atypical antipsychotics cause unfortunate adverse effects such as weight gain and some metabolic abnormalities resulting from weight gain such as insulin resistance, hyperlipidemia, hypoglycemia and cardiovascular diseases especially with the long term usage (Sertié et al., 2011). The case-control studies analyzing the possible reasons of weight gain such as food choice differences (Abbas and Liddle, 2013) or genetic studies regarding the polymorphisms in some genes such as histamine 1 receptor (*H1HR*) (Godlewska et al., 2013), regulators of glucose metabolism TBC1 domain family number 1 *(TBC1D1)* (Brandl et al., 2013), homozygotic C-allele polymorphisms in *MC4R* (Czerwensky et al., 2013) and the *NPY* (Tiwari et al., 2013) have been studied widely. However, the precise mechanism of the weight gain due to antipyschotic drug treatment remains to be elucidated and clarified.

One of the atypical antipsychotic drugs, olanzapine is known to have a potent effect on weight gain, specifically the highest weight gain recorded after clozapine (De Hert et al., 2011). Olanzapine exerts antagonistic effects on D2 (dopamine 2) and 5-HT_{2C} receptors and also have affinities for 5-HT_{2A}, 5-HT_6, D1–4, histaminergic H_1, adrenergic α-1 receptors. Other binding interactions include moderate bindings to 5-HT_3 and muscarinergic receptors, M_{1-5} and weak bindings to $GABA_A$ and 5-HT_{1B} receptors (Bymaster et al., 1996; Schmidt et al., 2001).

The mechanism of weight gain caused by atypical antipsychotics may be related to the other receptor binding affinities which also require further investigation (Kroeze et al., 2003) (Jin et al., 2008; Kirk et al., 2004). For instance, appetite is under the control of various peripheral and central nervous system hormones such as leptin, insulin,

Abbreviations: AgRP, agouti-related peptide; ARC, arcuate nucleus; CART, cocaine and amphetamine regulated transcript; NPY, neuropeptide Y; POMC, proopiomelanocortin; α-MSH, alpha melanocyte stimulating hormone; C_T, threshold cycle; ELISA, enzyme linked immunosorbent assay; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; qRT-PCR, quantitative real time polymerase chain reaction; 5HT, (5-hydroxytryptamine) serotonin; $5HT_{2C}$, serotonin 2C receptor.

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ghrelin and peptide YY (PYY) (Gale et al., 2004). There are a number of regulators of food intake such as leptin, which controls energy expenditure and food intake by affecting the neurons found in the ARC region of hypothalamus, namely proopiomelanocortin (POMC) and cocaine and amphetamine-regulated transcript (CART) which are anorexigenic, and neuropeptide Y (NPY) and agouti-related peptide (AgRP) which are orexigenic peptides (Konturek et al., 2005; Schwartz et al., 2000). At the time of satiety, leptin crosses blood-brain-barrier (BBB) and finds its receptor (Ob-Rb) on those neurons, causing an elevation of POMC and CART and reduction of NPY and AgRP synthesis and secretion (Jequier, 2002). POMC exerts its effects on feeding mechanism through the action of α-melanocyte stimulating hormone (α-MSH), the anorexigenic product of POMC, on melanocortin receptors (MC4R/MC3R). POMC neuronal group have 5-HT_{2C} (Heisler et al., 2002) and Ob-Rb receptors (Cheung et al., 1997) on their cell body, therefore the synthesis and secretion of POMC and the products of POMC are under the control of the stimulation of those receptors by serotonin and leptin. Thus, both serotonin and leptin lead to the activation of POMC neurons.

In this study, both the hypothalamic expression alterations and the peripheral levels of the neurohormones NPY, AgRP, CART and POMC together with the circulating leptin levels were determined to interpret the underlying mechanism of olanzapine induced weight gain in male rats. In our previous study, we have analyzed male psychotic patients' plasma levels of the candidate genes that were treated with olanzapine. In that study, 22 male patients were prescribed with olanzapine for 4 weeks. In these patients, BMI and blood lipid levels increased and there were alterations in plasma levels of candidate genes (Ak et al., 2013). Therefore, in the present study, the male rats were analyzed for their both plasma and the hypothalamic gene expression levels in the present study. It was hypothesized that the antagonistic effect of olanzapine on 5-HT_{2C} receptor might alter the expression of POMC; thereby, leading the de-regulation of the balance in the expression levels of the neurohormones; that are involved in the food intake and appetite.

2. Materials and methods

2.1. Animal groups

Olanzapine was administered to male Wistar-rats for 4-weeks. Four-weeks-old 24 male Wistar rats (100–130 g) were kind gift from the Department of Laboratory Animals, GMS (Gulhane Medical School), Ankara, Turkey. The rats were randomly divided into two groups as the vehicle group (n = 10) which was used as the control and the treatment group (n = 14). They were group-housed three per cage, under a 12-h light/dark cycle (lights on at 7 a.m.), in a room maintained at a temperature of 25 ± 2 °C and a humidity of 60%, with free access to water and 200 g/day normal rat chow with unlimited water.

2.2. Preparation and administration of the drug solution

Olanzapine was a generous provision from Ali Arif Ilac Sanayi (ARIS), İstanbul, Turkey. It was dissolved in 10% sucrose solution, and then the administration of 1 mL solution was performed orally by the help of a syringe. Each rat received olanzapine at a dose of 4 mg/kg/day for 4-weeks. Since the half-life of olanzapine in rats is 4–6 h (Aravagiri et al., 1999) the drug administration was carried out twice a day at 10 a.m. and at 6 p.m. in half doses, (2 mg/kg/dose). The vehicle group received only 1 mL of 10% sucrose solution as the control group.

2.3. Analyzing weight gain and food consumption

For the weight gain analysis, all rats were individually weighed every week in the morning and their weight was documented. For the analysis, the mean weights of rats for each week were normalized with respect to the mean of their initial weights and Olanzapine group was compared with vehicle group for each week.

Food consumption was analyzed as such that 200 g of food was given to the rats in a day and the remaining food was weighed the next day; consequently, the difference between two days was recorded as the amount of food consumed per cage. For the calculations, food consumed per cage was divided to rat numbers in that cage and food consumption per rat was evaluated. The values are given as mean ± standard error.

2.4. Plasma preparation and enzyme-linked immunosorbent assay (ELISA)

The animals were sacrificed by decapitation using the guillotine. All sacrifice procedures were completed in the morning (9:00 a.m.). The trunk blood was collected into the EDTA blood collection tubes and immediately centrifuged at 14,000 rpm for 10 min to obtain plasma. The plasma samples were aliquoted to prevent freeze-thaw cycles and stored at −80 °C until used.

Plasma concentration of the candidate peptides and leptin were determined by ELISA (Phoenix Pharmaceutical, Germany) according to manufactures protocols. The results were read at 450 nm by ELISA plate reader (SPECTRAmax 340PC, USA). The samples were run in duplicates.

2.5. Total RNA isolation

The hypothalamus (approximately 4 × 4 mm) was first extracted after the removal of whole brain from the skull. The brain was washed with phosphate buffered saline (PBS) (Sigma Aldrich, Germany) to prevent drying. The extracted whole hypothalamic samples were incubated in RNAlater™, RNA stabilization reagent (Qiagen, GmbH., Germany) overnight at 4 °C and then stored at −80 °C until used.

The RNA isolation was performed by TRI-reagent (Sigma Aldrich, Germany) (Chomczynski, 1993) with some modifications. Briefly, the hypothalami were washed with PBS and they were homogenized with the glass-teflon homogenization system in TRIreagent. After centrifugation at 12.000 ×*g* for 10 min., the supernatant was transferred into 200 μL ice-cold chloroform (Sigma Aldrich, Germany), and centrifuged at 12.000 ×*g* for 15 min two times. 500 μL ice-cold ice-cold isopropanol (Sigma Aldrich, Germany) was added. The mixture was incubated with 2 μL glycogen at −20 °C for 40 min. Following the incubation and centrifugation the pellet was washed with 75% ice-cold ethanol (Sigma Aldrich, Germany) and centrifuged then it was dissolved in nuclease free water. The RNA samples were treated with DNase-I by using DNA-free ™ Kit (Ambion, Invitrogen, Germany) to get pure RNA samples. The concentrations of the RNAs were measured using Nanodrop (Thermo Scientific, US) and the agarose gel electrophoresis was performed to check the integrity of RNA samples (Supplementary Figs. 1 and 2).

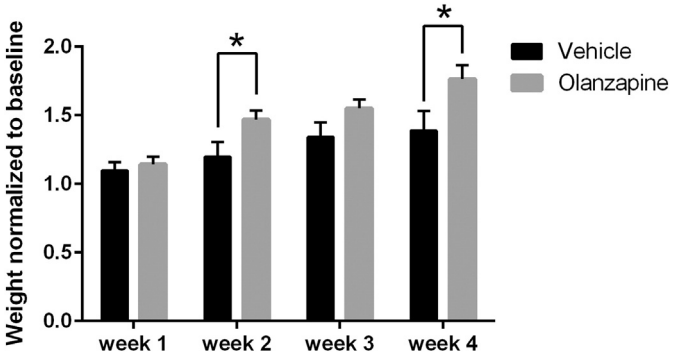

Fig. 1. Normalized weight of rats with respect to their initial weights during four weeks of olanzapine treatment. The olanzapine treated group showed significant weight gain at the end of the second and the fourth weeks (*$p < 0.05$).