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Research Report

# Effects of risperidone treatment on the expression of hypothalamic neuropeptide in appetite regulation in Wistar rats

Canan Kursungoz[a,1], Mehmet Ak[b,2], Tulin Yanik[a,*]

[a]Middle East Technical University, Department of Biological Sciences, Ankara, Turkey
[b]Department of Psychiatry, Gulhane School of Medicine, Ankara, Turkey

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ABSTRACT

Although the use of atypical antipsychotic drugs has been successful in the treatment of schizophrenia, they can cause some complications in the long-term use, including weight gain. Patients using these drugs tend to disrupt treatment primarily due to side effects. The atypical antipsychotic mechanism of action regulates a number of highly disrupted neurotransmitter pathways in the brains of psychotic patients but may also cause impairment of neurohormonal pathways in different brain areas. In this study, we investigated the circulating levels of hypothalamic neurohormones, which are related to appetite regulation; neuropeptide Y (NPY); alpha melanocyte stimulating hormone (α-MSH); cocaine and amphetamine regulated transcript (CART); agouti-related peptide (AgRP); and leptin in male Wistar rats, which were treated with risperidone, a serotonin antagonist, for four weeks. Alterations in the mRNA expression levels of these candidate genes in the hypothalamus were also analyzed. We hypothesized that risperidone treatment might alter both hypothalamic and circulating levels of neuropeptides through serotonergic antagonism, resulting in weight gain. Gene expression studies revealed that the mRNA expression levels of proopiomelanocortin (POMC), AgRP, and NPY decreased as well as their plasma levels, except for NPY. Unexpectedly, CART mRNA levels increased when their plasma levels decreased. Because POMC neurons express the serotonin receptor ($5HT_{2C}$), the serotonergic antagonism of risperidone on POMC neurons may cause an increase in appetite and thus increase food consumption even in a short-term trial in rats.



Abbreviations: AgRP, agouti-related peptide; ARC, arcuate nucleus; CART, cocaine and amphetamine regulated transcript; CNS, central nervous system; Ct, threshold cycle; ELISA, enzyme linked immunosorbent assay; EPS, extrapyramidal side effects; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; NPY, neuropeptide Y; POMC, proopiomelanocortin; qRT-PCR, quantitative real time polymerase chain reaction; 5HT, serotonin; $5\text{-}HT_{2C}$, serotonin receptor; α-MSH, alpha melanocyte stimulating hormone

*Corresponding author. Tel.: +90 312 210 6465; fax: +90 312 210 7976.
*E-mail addresses:* ckursungoz@gmail.com (C. Kursungoz), drmehmetak@gmail.com (M. Ak), tyanik@metu.edu.tr (T. Yanik).
[1]Tel.: +90 312 210 7620; fax: +90 312 210 7976.
[2]Tel.: +90 312 304 4501/06; fax: +90 312 304 4507.

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## 1. Introduction

Compared to typical antipsychotics, the discovery of atypical antipsychotics with minimum extrapyramidal side effects (EPS) and the successful treatment of both positive and negative symptoms of schizophrenia have resulted in a wider use of these drugs. However, despite the successful treatment of schizophrenic symptoms, atypical antipsychotics have important metabolic side effects, including weight gain or obesity, hypertension, diabetes mellitus, and changes in the lipid profile (McIntyre et al., 2001; Fleischhacker et al., 2008; De Hert et al., 2009; Maayan and Correll, 2010). Excessive weight gain during treatment may cause poor medication compliance; thus, patients may disrupt their treatment in long-term uses (Babiker, 1986). Although many investigations have been performed, the precise mechanisms of weight gain related to antipsychotics remain to be elucidated (Baptista, 1999; Correll et al., 2011; Yan et al., 2013; Yanik et al., 2013).

One of the atypical antipsychotic drugs, risperidone, has been associated with a moderate weight gain effect (Allison et al., 1999; Wetterling, 2001; Bobes et al., 2003; Müller et al., 2004; Lieberman et al., 2005; Bushe et al., 2013). Previous studies have shown that patients gained 2 and 8.64 kg after ten weeks (Allison et al., 1999) and 6-months treatment (Kelly et al., 1998), respectively. Risperidone is a potent antagonist for serotonin (5-HT) and dopamine receptors (Gardner et al., 2005), and it exhibits a selective monoaminergic antagonism on 5-$HT_2$ receptors (Ki of 0.12 to 7.3 nM) (Miyamoto et al., 2005).

Many animal studies have been performed to understand the mechanism of weight gain induced by risperidone. For example, when risperidone was injected into rats, it caused an increase in food intake and body weight gain in a dose dependent manner, as well as changes in leptin gene expression in adipose tissue at the lowest dose of administration (0.005 mg/kg). However, the same study also showed that the highest concentration of risperidone (0.5 mg/kg) resulted in a reduction in body weight in rats, which was explained as the interference of stress conditions induced by daily injection (Ota et al., 2002). Subcutaneous and intraperitoneal administration of risperidone in female rats resulted in an increase in body weight (Baptista et al., 2002 and Fell et al., 2004); however, this was not observed in male rats, which could indicate that gender difference may be important for weight gain (Baptista et al., 2002). In mice, when risperidone was mixed in peanut butter, food intake increased and activity levels decreased, resulting in an increase in body weight (Cope et al., 2009).

Few studies have analyzed cellular targets of the energy center of the brain, the hypothalamus. Fadel et al., 2002 found increased c-Fos expression in the orexin neurons at the lateral hypothalamic/perifornical area (LH/PFA) when rats were treated with atypical antipsychotics such as clozapine, olanzapine, chlorpromazine, and risperidone which cause significant weight gain but when rats were treated with antipsychotics such as ziprasidone, haloperidol, and fluphenazine which do not cause weight gain, c-Fos expression was not increased in those neurons. Another study showed that the three-week risperidone treatment of rats did not alter the expression of the leptin receptor and appetite-regulating peptides in the hypothalamus (Ota et al., 2005). As an evolutionarily old brain region, the hypothalamus consists of many nuclei, and functions as the primary central nervous system (CNS) component in the regulation of energy homeostasis (Rohner-Jeanrenaud et al., 1996; Sapolsky, 2013). One of these nuclei is the arcuate nucleus (ARC) which is the principal component for the regulation of food intake in which anorexigenic neurohormones, namely proopiomelanocortin (POMC), cocaine and amphetamine regulated transcript (CART) and orexigenic neurohormones, neuropeptide Y (NPY), agouti related peptide (AgRP) are synthesized and secreted (Funahashi et al., 2000, Konturek et al., 2005). These neuropeptides are modulated by leptin and insulin, which is responsible for decreasing appetite, thereby reducing food intake by decreasing *NPY* and *AgRP* and increasing *POMC* and *CART* expression in the hypothalamus (Campfield et al., 1995; Jeanrenaud and Rohner-Jeanrenaud, 2001; Kim et al., 2013). Previous studies have shown that POMC neurons, which represent the melanocortin system, can be stimulated using a 5-$HT_{2C}$ receptor agonist (Heisler et al. 2002). *POMC* exerts its effects on feeding through its anorexigenic product alpha melanocyte-stimulating hormone (α-MSH) through melanocortin receptors (Pritchard et al., 2002). In this study, we hypothesized that risperidone, through its 5-$HT_{2C}$ antagonism on the POMC neurons can alter the mRNA expression levels of candidate genes; thus, food intake may increase resulting in weight gain in rats. Moreover, recent data have shown consistent findings to our risperidone-treated schizophrenic patient study with regard to the plasma levels of leptin, NPY, α-MSH, and CART (Yanik et al., 2013).

## 2. Results

The grams of food consumption (Fig. 2a) and weight (Fig. 2b) of the rats showed that the risperidone group gained more weight (91.58 ± 5.84 g) compared to the vehicle group (72.30 ± 10.59 g) at the end of four week treatment and consumed more food. The body weight measurements showed that the risperidone group gained weight significantly at the end of the first ($^{*}p<0.05$) and the fourth weeks ($^{***}p<0.001$) compared to the vehicle group (Fig. 2b). The trend line of consumed food (g) versus days of the experiment between the groups indicated that the risperidone group had $y=1.581$ and the vehicle group had $y=0.336$ (Fig. 2a). This finding indicated that chronic risperidone administration triggered hyperphagic behavior especially in the first week of the treatment, and thus, the rats were excessively eating.

The hypothalamic RNA samples were isolated to determine the expression of candidate genes. The $A_{260}/A_{280}$ values ranged between 1.80–2.00 (Suppl. Table 1) and agarose gel images (Suppl. Fig. 1) corroborated the absence of DNA in the RNA samples. The expression levels of the candidate genes for appetite regulation were shown as the fold change in Fig. 3. These results revealed that there was a significant difference between the relative mRNA expression levels of *POMC* and *NPY*, which decreased 3-fold and 2-fold, respectively, in the risperidone group versus the vehicle group ($^{*}p<0.05$ for both *POMC* and *NPY*). Furthermore, *CART*