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Metabolic side effects induced by olanzapine treatment are neutralized by CB1 receptor antagonist compounds co-administration in female rats

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Abstract
Weight gain is an important side effect of most atypical antipsychotic drugs such as olanzapine. Moreover, although many animal models with metabolic side effects have been well defined, the interaction with other pathways has to be considered. The endocannabinoid system and the CB1 receptor (CB1R) are among the most promising central and peripheral targets involved in weight and energy balance. In this study we developed a rat model based 15-days treatment with olanzapine that shows weight gain and an alteration of the blood parameters involved in the regulation of energy balance and glucose metabolism. Consequently, we analysed whether, and by which mechanism, a co-treatment with the novel CB1R neutral antagonist NESS06SM, could attenuate the adverse metabolic effects of olanzapine compared to the reference CB1R inverse agonist rimonabant. Our results showed alterations of the cannabinoid markers in the nucleus accumbens and of orexigenic/anorexigenic markers in the hypothalamus of female rats treated with olanzapine. These molecular modifications could explain the excessive food intake and the resulting weight gain. Moreover, we confirmed that a co-treatment with CB1R antagonist/inverse agonist compounds decreased food intake and weight increment and restored all blood parameters, without altering the positive effects of olanzapine on behaviour. Furthermore, rimonabant and NESS06SM restored the metabolic enzymes in the liver and fat tissue altered by olanzapine. Therefore, CB1 receptor antagonist/inverse agonist compounds could be good candidate agents for the treatment of weight gain induced by olanzapine.

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1. Introduction

Despite the considerable advances achieved using second-generation atypical antipsychotic drugs for the treatment of schizophrenia, such as olanzapine, quetiapine, ziprasidone, and risperidone, many key aspects are still to be elucidated, such as some side effects. The most commonly reported side effect is weight gain (Cope et al., 2005). Patients affected by schizophrenia develop obesity, diabetes mellitus, hyperglycaemia, hypertension, and lipid abnormalities after administration of chronic atypical antipsychotics (Deng, 2013). Particularly, olanzapine, with antagonism/inverse agonism for 5HT2A and D2 receptors, is efficaciously prescribed to treat schizophrenia and a growing number of other psychiatric disorders in adults and children (Di Lorenzo and Brogli, 2010), but unfortunately its therapeutic benefits are overshadowed by an increased risk of developing obesity, diabetes mellitus, and lipid abnormalities (Di Lorenzo and Brogli, 2010). Moreover, although several other antipsychotics on the market do not induce weight gain, few of them have proved to be as therapeutically effective as olanzapine (Komossa et al., 2010). Hence, there is a great interest in developing a better treatment strategy in order to obtain a good therapeutic efficacy without increasing the body weight.

In the last ten years, among the compounds that induce weight loss and improve the metabolic parameters, neutral antagonists and inverse agonists of the CB1 receptor (CB1R) have been investigated (Mastinu et al., 2012; Meye et al., 2013). Despite the adverse effects of rimonabant, withdrawn from the European pharmaceutical market in 2009, the search for new CB1R antagonists, especially for peripherally acting neutral CB1R antagonists, was not abandoned (Nogueiras et al., 2008; Lazzari et al., 2012a, 2012b; Hsiao et al., 2015; Fulp et al., 2016). Particularly, the relevance of peripheral CB1R modulation for the liver and fat tissue metabolism activation with lack of side effects has been recently highlighted in various papers (Boon et al., 2014; Lu et al., 2016). In this context, we have recently synthesized and characterized a neutral CB1R antagonist compound, namely NESS06SM, that reduces body weight and improves the metabolic syndrome condition without inducing rimonabant side effects (Mastinu et al., 2013).

In this work, we have contrasted the metabolic side effects of olanzapine by a chronic co-treatment with an antipsychotic and NESS06SM or rimonabant. For this study we used female rats because they were shown to be more suitable for modelling antipsychotic-induced weight gain than male rats (Albaugh et al., 2006; Boyda et al., 2010; Davey et al., 2012; van der Zwaal et al., 2014). Then, body weight, food intake, and metabolic and behavioural parameters have been assayed to ascertain the beneficial effects of CB1R antagonists/inverse agonists on olanzapine side effects.

2. Experimental procedures

2.1. Chemicals

NESS06SM was synthesized according to previously reported procedures (Mastinu et al., 2013). Rimonabant and olanzapine were purchased by KEMPROTECH Limited, Middlesbrough, UK. Amphetamine was purchased by Tocris Bioscience, Bristol, UK.

2.2. Animals and treatment

The experiments were performed according to the UE guidelines (CEE N°86/609) for the care and use of experimental animals and were approved by Ethical Committee of the Institute of Translational Pharmacology, National Research Council, Italy.

Forty female wistar rats (6-7 week old, 100-150 g) were purchased from Charles River (Calco, Lecco, Italy). After one-week acclimation period, they were housed in pairs, in plastic cages kept at 23±2 °C, 55±15% relative humidity, and maintained on a reverse 12:12 h light/dark cycle. Animals were fed ad libitum with a normal weighted chow (D12450B, 10% fat, 70% carbohydrate, 20% protein, total 3.85 kcal/g; Research Diets Inc., New Brunswick). Food intake was measured by dividing the difference of the consumed food for the two rats in the cage. We have monitored oestrous cycle during the whole chronic treatment by visual and cytological analysis and we have observed a regular oestrus cycle. We randomly divided the rats into four groups balanced on body weight, with 8 rats per group. The groups were: control group treated with vehicle (VH, for five week), group treated with olanzapine 4 mg/kg/day (OLA, for five week), group treated with olanzapine 4 mg/kg/day and rimonabant 10 mg/kg (OLA+RIMO), and at last group treated with olanzapine 4 mg/kg/day and NESS06SM 10 mg/kg (OLA+N6SM). Rimonabant and NESS06SM were administrated at the end of the second week until the fifth week. The dosages were chosen according to previous data (Liebig et al., 2010; Lazzari et al., 2011; Mastinu et al., 2013). In order to reduce the stress of two gavages, only one operator administrated the drugs. All drugs were prepared daily, suspended in Tween 80 and 0.9% Saline, sonicated, and administered by oral gavage. Olanzapine, due to its short half-life in rodents (van der Zwaal et al., 2008), was administered twice a day. This dose was shown to be appropriate to model pharmacodynamic effects of olanzapine in rats as reported Kapur et al. (2003). In our unpublished data, rimonabant and NESS06SM showed a half-life of 7.5 and 8.9 h respectively, therefore cannabinoid compounds were administrated once a day. We daily measured the body weight, the body length and the food consumption.

2.3. Amphetamine-induced hyperlocomotion test

We have evaluated the motility of female rats during diestrum phase, following a modified version of the open field protocol by Gil-Ad et al. (2014). In order to evaluate the co-treatment effects of olanzapine and cannabinoid compounds, we treated the rats with a dopamine agonist. In particular, 90 min after the last drug administration the female rats were treated with 5 mg/kg/ip of D-amphetamine and after 20 min were placed 1 h into the testing room to habituate to the environment. After acclimating, each animal was placed in the centre of a rectangular black plastic box (60 × 60 × 50 cm). Their behaviour was recorded by a video camera for 30 min (10:00-10:30) and their locomotor activity was analysed by means of a tracking software system (ANY-maze software, Stoelting Co., USA). We evaluated the total distance travelled, the total time mobile and the speed, and we compared it to the control group, composed of 4 female rats treated with amphetamine and 4 with physiologic solution. Open field arena was frequently washed to remove olfactory cue between experiments with rats. Moreover, all the open field tests were performed under low lighting (100 lx).

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