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Effects of chronic antipsychotic drug exposure on the expression of Translocator Protein and inflammatory markers in rat adipose tissue

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ABSTRACT

The precise effect of antipsychotic drugs on either central or peripheral inflammation remains unclear. An important issue in this debate is to what extent the known peripheral metabolic effects of antipsychotics, including increased adiposity, may contribute to increased inflammation. Adipose tissue is known to contribute to the development of systemic inflammation, which can eventually lead to insulin resistance and metabolic dysregulation. As a first step to address this question, we evaluated whether chronic exposure to clinically comparable doses of haloperidol or olanzapine resulted in the immune activation of rat adipose tissue. Samples of visceral adipose tissue were sampled from male Sprague–Dawley rats exposed to, haloperidol, olanzapine or vehicle (all n = 8), for 8 weeks. From these we measured a cytokine profile, protein expression of F4/80 (a phenotypic macrophage marker) and translocator protein (TSPO), a target for radiotracers putatively indicating microgliosis in clinical neuroimaging studies. Chronic olanzapine exposure resulted in significantly higher adipose IL-6 levels compared with vehicle-controls (ANOVA $p = 0.008$, Bonferroni post-hoc test $p = 0.006$); in parallel, animals exposed to olanzapine had significantly higher F4/80 expression when compared with vehicle-controls (Mann Whitney Test, $p = 0.014$), whereas there was no difference between haloperidol and vehicle groups (Mann Whitney test, $p = 0.1$). There were no significant effects of either drug on adipose TSPO protein levels. Nevertheless, we found a positive correlation between F4/80 and TSPO adipose protein levels in the olanzapine-exposed rats (Spearman's rho = 0.76, $p = 0.037$). Our data suggest that chronic exposure to olanzapine, but not haloperidol, increases production of the pro-inflammatory cytokine IL-6 in adipose tissue and increased macrophages expression (F4/80), in the absence of measurable changes in TSPO with respect to vehicle. This may have potentially important consequences in terms of metabolic dysregulation associated with long-term antipsychotic treatment.

1. Introduction

Converging lines of evidence from genetics, *post-mortem* neuropathology, neuroimaging and peripheral biomarker studies suggest that at least a sub-set of patients with schizophrenia have elevated peripheral and central inflammation (Baumeister et al., 2014; Mondelli et al., 2017). Whether antipsychotic drug treatment has a confounding effect on these data remains controversial (Cotel et al., 2015; Baumeister et al., 2016).

In terms of peripheral circulating cytokines, clinical studies suggest mixed findings, with increases, decreases, or no change reported following antipsychotic treatment (Baumeister et al., 2016; Tourjman et al., 2013). Similar heterogeneity is found in those studies, which examined central (cerebrospinal fluid) cytokines, although these are much fewer in number (Baumeister et al., 2016). In the context of peripheral inflammatory markers, we have previously suggested that these discrepancies may, at least in part, depend on the peripheral metabolic side-effect profile of antipsychotics, specifically, increased weight gain and adiposity (Baumeister et al., 2016; Fonseka et al., 2016). For example, Song et al. (2014) investigated the effects of

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risperidone on plasma cytokine levels in patients with first episode schizophrenia who were initially antipsychotic drug naïve. They found an initial decrease in circulating interleukin (IL)1-β and IL-6 levels in the first weeks of treatment, followed by an increase in these cytokines back to pre-treatment levels after 6 months of treatment, which occurred in parallel with a steady weight gain (Song et al., 2014). These findings suggest that whilst the peripheral inflammation detected in these patients may initially decrease with antipsychotic treatment, it may thereafter increase as a function of the weight gain and increased adiposity induced by these drugs. It is generally acknowledged that treatment with second generation antipsychotics, such as olanzapine, is associated with higher weight gain and higher risk for developing diabetes compared with first generation antipsychotics, such as haloperidol (Smith et al., 2008; Newcomer, 2005). However, whether and how these two drugs (olanzapine and haloperidol) may have different metabolic effects associated to molecular mechanisms is still unclear. Importantly, adipose tissue is no longer considered a mere storage site for excess energy, as it was few decades ago, but it is now recognized to be an active endocrine organ involved in several metabolic and immune processes. It is therefore surprising that only a few studies to date have explored the effects of antipsychotic treatment on immune activation specifically in adipose tissue (Davey et al., 2012; Victoriano et al., 2010; Zhang et al., 2014).

Rodent models of chronic antipsychotic exposure using paradigms that achieve clinically comparable dosing and pharmacokinetics offer a means to investigate the molecular basis of these drug effects, dissociated from the disease process, something that is not easily achieved in clinical psychopharmacology studies (Amato et al., 2017). Using this approach, we have previously demonstrated that in male rats chronically exposed to haloperidol, or olanzapine, the former drug significantly increased the percentage of visceral adipose tissue, albeit in the absence of weight gain (Mondelli et al., 2013). Although the percentage of visceral adipose tissue was not affected by exposure to olanzapine, these rats developed abnormalities in insulin signaling pathways in the liver, specifically, decreased IRS2 levels, decreased GSK3α phosphorylation and elevated GSK3β, that were not observed in haloperidol or vehicle-exposed rats (Mondelli et al., 2013). These data suggest that even in the absence of antipsychotic-induced weight gain and increased adiposity, which is known to be sex-specific in rat models (Pouzet et al., 2003), chronic olanzapine exposure still induces the development of clinically relevant metabolic abnormalities in rat hepatic tissue (Mondelli et al., 2013).

One of the other main questions rising from the increasing evidence of peripheral inflammation in patients with psychosis is whether there is any link between peripheral and central inflammation in these patients. In humans, neuroinflammation is commonly assessed using positron emission tomography (PET) to measure the specific binding of radioligands to the translocator protein (TSPO). However, data on TSPO binding in patients with psychosis have shown so far inconsistent findings (Bloomfield et al., 2016; Mondelli et al., 2017; Notter et al., 2018a; Notter et al., 2018b). The reason behind this inconsistency is not clear, but it may reflect methodological differences in quantification of tracer binding across studies, illness stage-specific effects or exposure to antipsychotics. In terms of the latter possibility, Holmes and colleagues (Holmes et al., 2016) recently reported increased TSPO binding in patients with schizophrenia receiving depot antipsychotic medication, as compared to antipsychotic naïve patients of a similar age, body mass index and illness duration (Holmes et al., 2016). Notably, very few studies have investigated the expression of TSPO in adipose tissue as a putative index of its inflammatory state (Campioli et al., 2011; Thomson et al., 2013) and none has done so in patients with schizophrenia either on or off antipsychotic medication.

In this paper and with our rat model we aim to answer two new questions: First, what is the inflammatory state of adipose tissue in animals chronically exposed to antipsychotic treatment? Second, how does this relate to adipose TSPO expression? To address these, we measured cytokines and the protein levels of TSPO and F4/80, the latter an index of tissue macrophage infiltration, in frozen archival adipose tissue samples from animals chronically exposed to clinically-relevant doses of haloperidol and olanzapine, in which we have previously demonstrated the aforementioned hepatic metabolic abnormalities, increased adiposity, and brain microgliosis (Mondelli et al., 2013; Cotel et al., 2015).

2. Materials and methods

2.1. Animals

Twenty-four ten-week old male Sprague-Dawley rats (Charles River, Kent, UK), with initial body weight between 240–250 g, were housed in groups of four per cage in a 12 h light-dark cycle with food and water available *ad libitum*. Room temperature and humidity were both controlled (21 ± 2 °C the former, 55 ± 10% the latter). Animal procedures were conducted in compliance with local ethical approval and in accordance with the Home Office Animals (Scientific Procedures) Act 1986, UK.

2.2. Chronic drug administration

Animals were divided randomly into three treatment groups: common vehicle (n = 8), haloperidol (2 mg/kg/d s.c., n = 8), olanzapine (10 mg/kg/d s.c., n = 8). Vehicle (β-hydroxypropylcyclodextrin, 20% wt/vol, acidified by ascorbic acid to pH 6), haloperidol (2 mg kg^{-1} per day; Sigma-Aldrich, Gillingham, Dorset, UK) and olanzapine (10 mg kg^{-1} per day; Biophore Pharmaceuticals, Hyderabad, Andra Pradesh, India) were administered to the animals using osmotic minipumps for 8 weeks (approximately 5 human years, considering 11.8 rat days equals 1 human year) (Mondelli et al., 2013). The doses of each antipsychotic were chosen based on previous D2 receptor occupancy studies in our laboratory (Kapur et al., 2003; Vernon et al., 2011); serum-plasma levels achieved following chronic administration in this study reflect D2 occupancy in the range of 75–90% (Vernon et al., 2011), similar to the higher end of clinical exposure. The osmotic pump delivers at a steady rate in comparison to daily injections where drug levels fall to undetectable levels within 24 h (half-life < 2.5 h in rats for most antipsychotics). The osmotic minipumps (Alzet Model 2ML4, 28 days; Alzet, Cupertino, CA, USA) filled with drug or vehicle solutions were inserted subcutaneously on the back flank under isoflurane anesthesia (5% induction, 1.5% maintenance) and replaced once after 28 days. After 56 days (8 weeks) exposure, animals were sacrificed under terminal anaesthesia (sodium pentobarbital, 60 mg kg^{-1} intraperitoneal). Visceral adipose tissue samples were rapidly dissected, snap frozen in isopentane and stored at −80 °C. The data in the current study shows findings from these adipose samples, harvested from the above animals. Therefore, no new animals were generated for this study.

2.3. Multiplex cytokine assay

Visceral adipose tissue samples were prepared for cytokine detection immunoassay according to the following protocol: previously thawed and weighed samples were sonicated in ice-cold lysis buffer (10% glycerol, 1% Triton X-100, 5% HEPES, 150 mM NaCl, Pierce® protease inhibitor cocktail) and rotated at 4 °C for 30 min. The homogenates were then centrifuged at 18,000 *g* for 20 min at 4 °C. The supernatant was carefully pipetted from under the fatty layer and transferred to clean tubes. The electrochemiluminescence immunoassay was performed according to the manufacturer's instructions (V-Plex Plus Proinflammatory Panel 2 (rat) kit, Meso Scale Diagnostics, Rockville, Maryland, USA). Samples were diluted four-fold. The following cytokines were assessed in duplicates: IL1-β, IL-4, IL-5, IL-6, interferon (IFN)-γ, KC/GRO, and tumor necrosis factor (TNF)-α, with the