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Modulation of triglyceride accumulation in adipocytes by psychopharmacological agents *in vitro*

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Weight gain is a major problem during psychopharmacological treatment. Research has concentrated on the appetite inducing properties and mechanisms of these drugs in the central nervous system. The potential contribution of direct effects of drugs on metabolically relevant peripheral cells such as adipocytes is less well understood.

We examined the influence of the antidepressant imipramine, the antipsychotic clozapine, and the mood stabilizer lithium on preadipocytes and adipocytes *in vitro*, using Simpson-Golabi-Behmel syndrome (SGBS) cells, an established human preadipocyte model. Parameters of cell differentiation and signaling, and cell metabolism were measured.

We found significantly increased triglyceride accumulation in adipocytes after supplementation with imipramine and lithium at therapeutic concentrations, compared to non-supplemented control samples. However, gene expression levels of an early marker of adipogenesis, the peroxisome proliferator-activated receptor gamma (PPAR-γ) and a late marker of adipogenesis, the fatty acid binding protein 4 (FABP4), as well as expression of adiponectin (ADIPOQ) did not change significantly in the presence of these psychopharmacological agents.

The results suggest a direct influence of imipramine and lithium but not clozapine on fat storage of adipocytes. The underlying mechanisms of fatty acid storage and adipocyte differentiation however remain to be elucidated.

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1. Introduction

Weight gain is a crucial and frequent side effect of psychopharmacological treatment. Some drugs are associated with weight gains of up to 20 kg in susceptible individuals over several months of treatment (Mahendran et al., 2010; Marić et al., 2008). As a consequence, patients have an increased risk of diabetes mellitus, cardiovascular diseases and other adiposity- and overweight-related problems (Steffenhagen et al., 2012; Usher et al., 2013). Examples of psychopharmacological agents with a high risk of weight gain include the antipsychotic clozapine, tricyclic antidepressants such as imipramine, and the mood stabilizer lithium (Zimmermann et al., 2003; Himmerich et al., 2015).

Weight gain during psychopharmocological treatment is thought to be the consequence of changes in appetite regulation (Kluge et al., 2007). Pathophysiological mechanisms have been increasingly identified over the past decade. These include antihistaminergic drug effects (Kroeze et al., 2003), activation of hypothalamic adenosine monophosphate-activated protein kinase (AMPK) (Kim et al., 2007), modulation of ghrelin (Himmerich et al., 2005a, 2005b; Pinar et al., 2008; Zhang et al., 2013) and leptin (Atmaca et al., 2003; Schilling et al., 2013) signaling and changes in the production of cytokines and adipocytokines such as adiponectin (ADIPOQ) (Pinar et al., 2008), and the modulating impact of several genes, most of all melanocortin 4 receptor (MC4R),

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serotonin 2C receptor (HTR2C), and leptin, neuropeptide Y (NPY) and cannabinoid receptor 1 (CNR1) genes (Müller et al., 2009).

Metabolic disturbances during treatment with these drugs may occur independent of weight gain. For example, diabetes, impaired glucose tolerance and hypertriglyceridemia often appear during clozapine treatment even in the absence of weight gain (Steffenhagen et al., 2012; Zimmermann et al., 2003). Furthermore, antipsychotic drugs directly impair insulin action in adipocytes and have significant effects on glucose transport, lipogenesis, and lipolysis (Vestri et al., 2007). Therefore, psychopharmacological agents may directly influence metabolically relevant cells such as fat cells.

We recently showed in children (Landgraf et al., 2015), that accumulation of fat in the development of obesity not only results from hypertrophy, but to a great extent from hyperplasia of adipocytes. In addition to specialized lipid storing mature fat cells, adipose tissue contains several other cell types including preadipocytes. These preadipocytes retain their capacity to differentiate into mature adipocytes throughout the entire life span (Gregoire, 2001). Hence, hyperplasia through differentiation of preadipocytes into adipocytes is an important pathway in the accumulation of body fat and the development of obesity.

The differentiation of preadipocytes into adipocytes is a highly regulated process involving a cascade of transcription factors. These direct the development of adipocytes with specific morphologic, metabolic, and endocrine features. The mechanisms regulating the differentiation of adipocytes are not yet understood in detail. However, important markers of adipocyte differentiation have been described, for example peroxisome proliferator-activated receptor gamma (PPAR-γ), an early marker of adipogenesis (Lowell, 1999), and fatty acid binding protein 4 (FABP4) a late marker of adipogenesis (Furuhashi et al., 2015). Important functions of adipocytes are regulated by the adipocytokine ADIPOQ, a signaling molecule (Fu, 2014) that modulates insulin sensitivity and fatty acid oxidation (Diez and Iglesias, 2003).

Past studies have examined specific psychopharmacological effects on adipogenesis, adipocyte metabolism and signaling produced by the antipsychotics clozapine and olanzapine (Hemmrich et al., 2006; Yang et al., 2007), the antidepressant phenelzine (Chiche et al., 2009) and lithium (Aratani et al., 1987). These studies have examined the effects of single drugs or drugs from one class. Systematic studies comparing different classes of psychopharmacological agents on a range of effects on adipocytes have not been reported.

Therefore, we examined the influence of imipramine, clozapine, and lithium on triglyceride accumulation, adipocyte differentiation and ADIPOQ expression *in vitro*. The respective agents represented the drug classes of tricyclic antidepressant, atypical antipsychotic, and mood stabilizer. We applied the psychopharmacological agents at therapeutically relevant concentrations in accordance with the AGNP-TDM expert group consensus guidelines for therapeutic drug monitoring in psychiatry (Baumann et al., 2004) to Simpson-Golabi-Behmel syndrome (SGBS) cells, an established human preadipocyte model (Wabitsch et al., 2001; Körner et al., 2005).

2. Methods and material

2.1. Cell culture of preadipocytes and adipocytes

The experiments were performed using Simpson-Golabi-Behmel syndrome (SGBS) cells (Wabitsch et al., 2001; Körner et al., 2005) supplied by M. Wabitsch (Ulm, Germany). Cells were cultured in basal SGBS medium consisting of DMEM/Ham F12 medium (Life Technologies, Karlsruhe, Germany) supplemented with 33 μM biotin and 17 μM pantothenic acid. Cells were differentiated into mature adipocytes as described by Körner (Körner et al., 2005). Briefly, SGBS preadipocytes were grown to confluence in basal medium supplemented with 10% FCS. Adipocyte differentiation was induced in a serum-free differentiation medium constituted by supplementing basal medium with 20 nM insulin, 0.2 nM triiodothyronine, 100 nM hydrocortisone, and 0.13 nM apo-transferrin. Additional additives on days 1–4 only were 2 μM rosiglitazone, 25 nM dexamethasone and 500 μM 3-isobutyl-1-methylxanthine (IBMX). Cells were harvested at day 12 post-induction.

2.2. Stimulation experiments

Psychopharmacological agents were added to the differentiation medium at maximum therapeutic concentrations (300 ng/ml imipramine or 600 ng/ml clozapine or 1.2 mM lithium-carbonate (Li_2CO_3)) based on the AGNP-TDM expert group consensus guidelines for therapeutic drug monitoring in psychiatry (Baumann et al., 2004). The agents were added for the entire 12-day differentiation period. The use of these concentrations is an established method for *in vitro* experiments (Himmerich et al., 2010, 2011; Petersein et al., 2015). Clozapine was dissolved in dimethyl sulfoxide (DMSO); accordingly a DMSO solvent control was included. Untreated cells served as control.

We also performed a concentration series for Li_2CO_3 adding 1.2, 2.4, 5 or 20 mM to the differentiation medium. At the 20 mM concentration the pH of the culture medium was affected, therefore a pH control sample was run with sodium carbonate at a concentration of 20 mM.

2.3. Quantification of triglyceride content

Adipocytes at day 12 post-induction were fixed in Roti®-Histofix 4% (Carl Roth, Karlsruhe, Germany), washed with phosphate buffered saline (PBS) and stained with Oil Red-O working solution (0.3% in 60% isopropanol) for 15 min. After washing with running water, Oil Red-O was extracted by incubation with isopropanol, and quantified at 540 nm using the FLUOstar OPTIMA (BMG LABTECH, Offenburg, Germany).

2.4. Analysis of mRNA expression

Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany) including on-column DNA digestion according to the manufacturer's instructions. 500 ng were reverse transcribed using M-MLV reverse transcriptase and random hexamer [p(dN)6] primers. Gene expression was quantified by TaqMan probe-based gene expression assay on the ABI 7500 Sequence Detection System (Applied Biosystems, Germany). For standardization of gene expression target genes, PPAR-γ, FABP4, and ADIPOQ were normalized to the mean of the three housekeeping genes TATA-box-binding protein (TBP), beta-actin (ACTB), and hypoxanthine phosphoribosyltransferase 1 (HPRT1). Sequence information of primers and probes are given in Table 1.

2.5. Statistical analyses

Data are presented as means ± SEM of at least 3 independent cell culture experiments, each performed in triplicate. Expression during adipogenesis was analyzed by one-way ANOVA with repeated measures and Dunnett post tests. The differences between means in stimulation experiments were analyzed by Student t-test. The threshold for statistical significance was set at $P < 0.05$. Statistical analyses were performed using Graph Pad Prism 5 (GraphPad Software Inc., San Diego, CA, USA).

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