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EXERCISE PREVENTS DOWNREGULATION OF HIPPOCAMPAL PRESYNAPTIC PROTEINS FOLLOWING OLANZAPINE-ELICITED METABOLIC DYSREGULATION IN RATS: DISTINCT ROLES OF INHIBITORY AND EXCITATORY TERMINALS

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Abstract—Schizophrenia patients treated with olanzapine, or other second-generation antipsychotics, frequently develop metabolic side-effects, such as glucose intolerance and increased adiposity. We previously observed that modeling these adverse effects in rodents also resulted in hippocampal shrinkage. Here, we investigated the impact of olanzapine treatment, and the beneficial influence of routine exercise, on the neurosecretion machinery of the hippocampus. Immunodensities and interactions of three soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins (syntaxin-1, synaptosome-associated protein of 25 kDa (SNAP-25) and vesicle-associated membrane protein (VAMP)), synaptotagmin and complexins-1/2 were quantified in the hippocampus of sedentary and exercising rats exposed over 9 weeks to vehicle ($n = 28$) or olanzapine (10 mg/kg/day, $n = 28$). In addition, brain sections from subgroups of sedentary animals ($n = 8$) were co-immunolabeled with antibodies against vesicular GABA (VGAT) and glutamate (VGLUT1) transporters, along with syntaxin-1, and examined by confocal microscopy to detect selective olanzapine effects within inhibitory or excitatory terminals. Following olanzapine treatment, sedentary, but not exercising rats showed downregulated (33–50%) hippocampal densities of SNARE proteins and synaptotagmin, without altering complexin levels. Strikingly, these effects had no consequences on the amount of SNARE protein–protein interactions. Lower immunodensity of presynaptic proteins was associated with reduced CA1 volume and glucose intolerance. Syntaxin-1 depletion appeared more prominent in VGAT-positive terminals within the dentate gyrus, and in non-VGAT/VGLUT1-overlapping areas of CA3. The present findings suggest that chronic exposure to olanzapine may alter hippocampal connectivity, especially in inhibitory terminals within the dentate gyrus, and along the mossy fibers of CA3. Together with previous studies, we propose that exercise-based therapies might be beneficial for patients being treated with olanzapine.

Key words: atypical antipsychotics, metabolic side-effects, SNARE, ELISA, confocal microscopy, inhibitory terminals.

INTRODUCTION

Second-generation antipsychotic drugs (SGAs) are commonly prescribed for the pharmacological management of psychosis and a wide range of other neuropsychiatric disorders (Procyshyn et al., 2010; McKean and Monasterio, 2012). Unfortunately, most SGAs are associated with metabolic side-effects, including weight gain, glucose dysregulation and dyslipidemia, which can lead to the development of metabolic syndrome, and ultimately Type 2 Diabetes Mellitus and cardiovascular disease (Leung et al., 2012; Weston-Green et al., 2013). Animal models of SGA-induced metabolic dysregulation have been invaluable in elucidating the physiological pathways contributing to glucose intolerance and insulin resistance, as well as increased adiposity (Boyda et al., 2010).

Consistent with numerous previous studies (Chintoh et al., 2008; Victoriano et al., 2009), we have shown that acute treatment with the SGA olanzapine causes profound glucose intolerance and insulin resistance in rats (Boyda et al., 2013). Previous research from our laboratory also noted that rats exposed to *chronic* treatment with olanzapine developed a sustained glucose intolerance

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Abbreviations: ANCOVA, analysis of covariance; ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; CA, *Cornu Ammonis*; cELISA, capture ELISA; dELISA, direct ELISA; DG, dentate gyrus; ELISA, enzyme-linked immunosorbent assay; GABA, gamma-aminobutyric acid; GCL, granule cell layer; IF, immunofluorescence; IP, immunoprecipitation; OD, optical density; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PVDF, polyvinylidene difluoride; SDS, sodium dodecyl sulfate; SGAs, Second-generation antipsychotic drugs; SNAP-25, synaptosome-associated protein of 25 kDa; SNARE, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor; t-SNARE, target-SNARE; TBS, Tris-buffered saline; v-SNARE, vesicle-SNARE; VAMP, vesicle-associated membrane protein; VGAT, vesicle GABA transporter; VGLUT1, vesicle glutamate transporter-1.

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and increased adiposity that lasted for the duration of SGA treatment (Boyda et al., 2012, 2014). Intriguingly, rats subjected to this chronic olanzapine regimen showed smaller hippocampal volumes at the end of the study compared to vehicle-treated animals (Barr et al., 2013). Of note, glucose dysregulation and chronic hyperglycemia have been associated with hippocampal atrophy in clinical studies (Convit, 2005; McCrimmon et al., 2012). Interestingly, when animals treated chronically with olanzapine were allowed to exercise daily during the treatment, both glucose intolerance and loss of hippocampal volume were prevented (Barr et al., 2013; Boyda et al., 2014). These findings are in agreement with the observed beneficial effects of exercise-based therapies in schizophrenia patients (Malchow et al., 2013; Hjorth et al., 2014; Rosenbaum et al., 2014), which include increases in hippocampal volume (Pajonk et al., 2010; Erickson et al., 2011; Takahashi et al., 2012; but see also Scheewe et al., 2013).

Among the potential physiological substrates that may underlie the effects of olanzapine on hippocampal volume, proteins that regulate synaptic connectivity and exocytosis of synaptic transmission are of particular interest. Docking and fusion of vesicles at the presynaptic nerve endings, and subsequent neurotransmitter release, are engineered by the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) (Söllner et al., 1993). The interaction between three molecules yields the SNARE heterotrimer: syntaxin-1, and synaptosome-associated protein of 25 kDa (SNAP-25), preassembled into the target (t-)SNARE within the presynaptic membrane, and the vesicle-associated membrane protein (VAMP, or synaptobrevin), conforming vesicle (v-)SNAREs. Previous postmortem studies have observed that schizophrenia is associated with changes in the amount of SNARE and SNARE-associated proteins, such as the complexins, in the hippocampus (Young et al., 1998; Harrison, 2004).

Exposure to antipsychotic drugs might regulate (directly or indirectly) the expression and/or function of components within the neurosecretion machinery in humans and rodents. A recent postmortem brain study showed that the immunodensities of syntaxin-1, VAMP and the SNARE complex, but not SNAP-25, were reduced in the prefrontal cortex of antipsychotic-medicated compared to drug-free schizophrenia-matched cases (Gil-Pisa et al., 2012). Pharmacological studies have reported contrasting effects of first- and second-generation antipsychotics in rodent brain (summarized in Table 1). However, none of these studies was performed in the context of the metabolic side-effects of SGAs, and few addressed hippocampal alterations.

Against this background, the present study explored the impact of a chronic regimen of olanzapine treatment with known and precisely quantified metabolic side-effects on the hippocampal expression and function of presynaptic proteins involved in SNARE complex assembly. In addition, the potential benefits of routine physical activity concurrent with olanzapine treatment were also investigated. To this end, we used quantitative immunoassays to measure the levels of SNARE proteins, synaptotagmin and complexins 1 and 2, as well as the amount of SNARE protein–protein associations (as a functional outcome), in the hippocampus of sedentary and exercising rats chronically treated with olanzapine. Finally, we used confocal microscopy to address possible selective effects of the treatments targeting inhibitory and/or excitatory presynaptic terminals.

EXPERIMENTAL PROCEDURES

Animals and treatments

The study was conducted with brain tissue retained from our previous study of the metabolic side-effects of chronic treatment with olanzapine (Boyda et al., 2014). Adult female Sprague–Dawley rats (Charles-River, Montreal, QC, Canada) were used, as females provide a more reliable and reproducible model to study SGA-induced metabolic side-effects than males (Boyda et al., 2010; Davey et al., 2012). Animal procedures were in compliance with the Canadian Council on Animal Care and NIH Guidelines for the Care and Use of Laboratory Animals. Experimental protocols were reviewed and approved by University of British Columbia's Animal Care Committee. Rats were housed in pairs under temperature (22 ± 1 °C) and humidity- (60%) controlled conditions, with a 12-h light–dark cycle. Food and water were provided *ad libitum*. Before treatments, rats were acclimated to the animal facilities for one week, and handled daily to reduce stress.

All animal procedures were described in detail previously (Boyda et al., 2012, 2014). Briefly, rats received daily vehicle (50% polyethylene glycol, and 10% ethanol; $n = 28$) or olanzapine (Toronto Research Chemicals, Toronto, ON, Canada; 10 mg/kg; $n = 28$) subcutaneous (s.c.) injections every Monday–Friday, followed by a 48-h washout period over the weekend, so that weekly glucose tolerance tests could be completed each Monday morning to assess glucose sensitivity. This treatment block was repeated over nine cycles. At the beginning of the study, animals were allocated into sedentary [vehicle- ($n = 9$) or olanzapine-($n = 10$) treated] or exercising [vehicle- ($n = 19$) or olanzapine-($n = 18$) treated] subgroups. Rats in the exercise subgroup were individually placed in an activity wheel (Med-Associates Inc., St Albans, VT, USA) and allowed to run freely for 1 or 3 h prior to drug treatment, while monitoring the distance covered. As there were no differences in the metabolic effects of 1 versus 3 h of exercise, nor difference in hippocampal volume, the groups were combined to increase statistical power.

Rats were euthanized with an intraperitoneal sodium pentobarbital overdose (130 mg/kg). Brains were immediately removed, and the hemispheres were separated and pseudo-randomly allocated for quantitative immunoassays or immunohistochemistry. Hemispheres chosen for immunoassays were rinsed in ice-cold artificial cerebrospinal fluid and frozen at −80 °C, while contralateral ones were fixed in 4% paraformalde hyde-phosphate-buffered saline (PBS) solution for 48 h at room temperature, and used for immunohistochemical assessments (Barr et al., 2013).