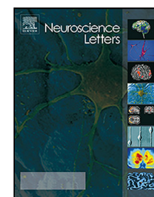
Neuroscience Letters xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet



Research article

Effects of haloperidol and clozapine administration on oxidative stress in rat brain, liver and serum

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HIGHLIGHTS

- Oxidative stress measured in brain, liver and serum following antipsychotics.
- Haloperidol increased levels of antioxidant peroxiredoxin-6 in frontal cortex.
- Clozapine increased lipid peroxidation in liver.
- Differences are consistent with documented adverse effects.

ARTICLE INFO

Article history:
Received 8 September 2014
Received in revised form 8 January 2015
Accepted 11 February 2015
Available online xxx

Keywords:
Antioxidant
Antipsychotic agent
Lipid peroxidation
Peroxiredoxin
Schizophrenia

ABSTRACT

Antipsychotics remain the standard of care for individuals with schizophrenia, despite their association with adverse effects including extrapyramidal symptoms, metabolic syndrome and agranulocytosis. While the biological mechanisms underlying these side effects remain unresolved, it has been proposed that oxidative stress may play a role in their development. The aim of this study was to evaluate markers of oxidative stress associated with first- and second-generation antipsychotics, focusing on protein and lipid oxidation and expression of the antioxidant proteins peroxiredoxin-2 and peroxiredoxin-6. Following 28-day administration of haloperidol, clozapine or saline to adult rats, brain grey matter, white matter, serum and liver samples were obtained and lipid peroxidation, protein oxidation, peroxiredoxin-2 and peroxiredoxin-6 levels quantified. In grey matter, peroxiredoxin-6 was significantly increased in the haloperidol-exposed animals, with a trend towards increased lipid peroxidation also observed in this group. In liver, lipid peroxidation was increased in the clozapine-exposed animals, with a similar trend noted in the haloperidol group. Antipsychotics did not produce significant changes in serum or white matter. Our results suggest that haloperidol and clozapine may induce oxidative stress in brain and liver, respectively, consistent with the documented adverse effects of these agents.

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1. Introduction

Schizophrenia is a complex mental disorder with an often chronic and disabling prognosis. Antipsychotic medications are standard care for individuals with schizophrenia and can be divided into first-generation antipsychotics (e.g. haloperidol) and second-generation antipsychotics (e.g. clozapine). One of the major concerns with antipsychotic medications is the frequency and severity of side effects. Extrapyramidal symptoms such as dystonia, parkinsonism, dyskinesia and akathisia are more prevalent in patients using first-generation antipsychotics, while weight gain and metabolic syndrome are associated with second-generation medications [1,2]. Metabolic syndrome is characterised by the occurrence of several abnormal metabolic findings including glucose intolerance, visceral obesity, hypertension, and dyslipidemia, and is associated with increased risk of cardiovascular disease [2]. In addition, clozapine can induce agranulocytosis, the decreased production of white blood cells, in a minority of patients [3].

Although the biological mechanisms underlying these adverse effects remain to be elucidated, it has been hypothesised that

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http://dx.doi.org/10.1016/j.neulet.2015.02.028
0304-3940/© 2015 Published by Elsevier Ireland Ltd.

oxidative stress may play a role. Oxidative stress has previously been linked to metabolic syndrome, agranulocytosis, and extrapyramidal symptoms including tardive dyskinesia, a movement disorder associated with prolonged exposure to antipsychotics e.g. [4–6]. Production of reactive oxygen species occurs naturally during aerobic metabolism. Under normal circumstances the body can defend itself against reactive oxygen species by upregulating the production of antioxidant enzymes. However, oxidative damage to lipids, proteins and DNA can occur when the amount of reactive oxygen species surpasses the capacity of the antioxidant defence system. The brain is particularly vulnerable to oxidative stress due to its substantial oxygen use, low production of antioxidants and high lipid levels [7].

In addition to a role in antipsychotic-associated adverse events, oxidative stress has also been implicated in the pathophysiology of schizophrenia [8–10]. In order to distinguish the relative contribution of antipsychotic treatment from disease process, we quantified several measures of oxidative stress following exposure to haloperidol or clozapine in a rat model. We specifically focussed on assessing tissue distribution of lipid and protein oxidation and quantification of two antioxidant enzymes, peroxiredoxin-2 and peroxiredoxin-6. While it has been reported that lipid peroxidation [11–18] and protein oxidation [11–13] are altered in rat brain following antipsychotic administration, data are inconsistent and vary depending on the specific brain region, time course, and drug studied. The effects of antipsychotics on white matter have not yet been determined, although given the substantial lipid content of white matter, this region may be highly sensitive to lipid peroxidation. Furthermore, decreased activities of the antioxidant enzymes superoxide dismutase and catalase have been reported in rat brain following antipsychotic administration in some [14–16], although not all [11,12] studies. While we, and others, have previously identified altered expression of peroxiredoxin-2 and peroxiredoxin-6 in post-mortem cerebral cortex in schizophrenia and bipolar disorder [19–21], whether antipsychotics influence peroxiredoxin protein levels remains to be elucidated. Finally, it is not clear whether antipsychotic-induced oxidative damage is specific to the brain, although reports of agranulocytosis, metabolic syndrome and hepatotoxicity following clozapine treatment [4,5,22] suggest that other organs, such as blood and liver, may also be affected.

2. Materials and methods

2.1. Animals and drug administration

Q3 Adult male Sprague–Dawley rats (Charles River, Montreal, Canada) were pair housed, with access to food and water *ad libitum*. Rats were pseudo-randomly divided into three groups ($n = 10$ per group) balanced for starting weight (270–320 g range), and administered haloperidol (1 mg/kg), clozapine (20 mg/kg) or vehicle (saline) daily for 28 days by intraperitoneal (i.p.) injection, as previously described [23]. All procedures were approved by the University of British Columbia Animal Care Committee and were conducted in accordance with the Canadian Council on Animal Care guidelines.

2.2. Tissue collection and sample preparation

Animals were sacrificed by decapitation 24 h after the final dose. Trunk blood was collected in Vacutainer tubes (BD, Franklin Lakes, NJ), left to clot for 30 min and centrifuged at $1100 \times g$ for 20 min. The recovered serum was stored at −70 °C until use. Liver samples were removed and rapidly frozen on dry ice. Brains were quickly removed, cooled in artificial cerebrospinal fluid and dissected as previously described [20]. Medial prefrontal cortex was dissected as areas Cg3, Cg1 and Fr2 [24]. Frontal white matter included the corpus callosum, cingulum bundle and external capsule. Tissue was dissected bilaterally, and tissue from both hemispheres combined. Dissected tissue was rapidly frozen on dry ice and stored at −70 °C until required. Tissue was homogenised in 10 volumes of ice-cold tris buffered saline (TBS) using a pellet pestle motor until an even suspension was obtained. Total protein concentrations were determined using a DC Protein Assay kit (BioRad Hercules, CA).

2.3. Measurement of lipid peroxidation

Malondialdehyde (MDA), a marker of lipid peroxidation, was quantified in serum and liver samples using a TBARS assay kit (Cayman Chemical, Ann Arbor, MI). Assays were run in duplicate, with between run correlations $r > 0.82$. For liver samples, MDA content was normalised against tissue wet weight. TBARS assays had poor reproducibility in brain tissue due to low signal and therefore levels of the lipid peroxidation marker 4-hydroxynonenal (4-HNE) were instead assessed in white and grey matter by western blotting. Briefly, for each sample, 10 μg of total protein was separated on 10% polyacrylamide gels, transferred to polyvinylidene fluoride (PVDF) membrane, and stained for total protein using the Pierce Reversible Protein Stain (Thermo Scientific, Rockford, IL). Blots were immunostained using a primary antibody against 4-HNE (1:500, Santa Cruz Biotechnology, Dallas, TX) and signal visualised with chemiluminescent substrate (ECL, GE Healthcare, Pittsburgh, PA) using a LAS-3000 Analyzer (FujiFilm, Tokyo, Japan). Images were quantified using Science Lab Image Gauge software, (FujiFilm, Tokyo, Japan). Staining intensity was normalised both to the average band intensity of three pooled standards run on each gel (to account for blot-to-blot variability) and to the total protein stain for that lane (to account for variations in sample loading).

2.4. Measurement of protein oxidation

Oxidised proteins were detected using an OxyBlot protein oxidation detection kit (EMD Millipore, Billerica, MA) performed according to the manufacturer's instructions with minor modifications. Briefly, for each sample 7.5 μg protein was incubated with either 2,4-dinitrophenylhydrazine (positive) or neutralisation solution (negative). Samples were then separated on 10% polyacrylamide gels followed by transfer to PVDF. Blots were immunostained using an antibody against DNP (1:2000, Sigma–Aldrich, St. Louis, MO), with signal visualised using chemiluminescent substrate. Proteins that underwent oxidative modification were identified as bands present in the derivatised sample, but not in the negative control sample. Staining intensity of derivatised samples was normalised to the total protein stain for that lane (to account for variations in sample loading), following subtraction of signal from adjacent negative control sample.

2.5. Measurement of peroxiredoxin-2 and peroxiredoxin-6

Western blotting was used to quantify expression of peroxiredoxin-2 and peroxiredoxin-6, as previously described [25]. Briefly, 4 μg protein per well, an amount determined to be within the linear range of detection, was separated using 10% polyacrylamide gels, then transferred to PVDF. Blots were stained for total protein and then immunostained using antibodies against peroxiredoxin-2 (1:10,000, Abcam, Toronto, ON) or peroxiredoxin-6 (1:1000–1:3000, Abcam, Toronto, ON). Staining intensity was normalised both to the average band intensity of three pooled standards run on each gel and to the total protein stain for that lane, as described above.

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