Chronic Antipsychotic Treatment in the Rat – Effects on Brain Interleukin-8 and Kynurenic Acid

Markus K. Larsson, Lilly Schwieler, Michel Goiny, Sophie Erhardt and Göran Engberg

Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden.

ABSTRACT: Schizophrenia is associated with activation of the brain immune system as reflected by increased brain levels of kynurenic acid (KYNA) and proinflammatory cytokines. Although antipsychotic drugs have been used for decades in the treatment of the disease, potential effects of these drugs on brain immune signaling are not fully known. The aim of the present study is to investigate the effects of chronic treatment with antipsychotic drugs on brain levels of cytokines and KYNA. Rats were treated daily by intraperitoneally administered haloperidol (1.5 mg/kg, $n = 6$), olanzapine (2 mg/kg, $n = 6$), and clozapine (20 mg/kg, $n = 6$) or saline ($n = 6$) for 30 days. Clozapine, but not haloperidol or olanzapine-treated rats displayed significantly lower cerebrospinal fluid (CSF) levels of interleukin-8 compared to controls. Whole brain levels of KYNA were not changed in any group. Our data suggest that the superior therapeutic effect of clozapine may be a result of its presently shown immunosuppressive action. Further, our data do not support the possibility that elevated brain KYNA found in patients with schizophrenia is a result of antipsychotic treatment.

KEYWORDS: interleukin-8, kynurenic acid, antipsychotic drugs, clozapine, chronic treatment

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CORRESPONDENCE: goran.engberg@ki.se

Introduction

Activation of the brain kynurenine pathway represents one of the most consistently found biochemical aberrations in schizophrenia and bipolar disorder with psychotic features. This pathway, representing the major route of tryptophan degradation, gives rise to neuroactive metabolites, eg, kynurenic acid (KYNA), an astrocyte-derived compound that preferably blocks *N*-methyl-D-aspartate (NMDA) and α7 nicotinic acetylcholine-receptors.[1–3] KYNA, and its precursor kynurenine, is increased in the cerebrospinal fluid (CSF) and postmortem brain in patients with schizophrenia and bipolar disorder with a history of psychosis.[4–9] Coherent with this, schizophrenia postmortem studies show increased activity of tryptophan 2,3-dioxygenase (TDO), a rate-limiting enzyme of this pathway, and also decreased expression and activity of kynurenine monooxygenase (KMO).[10]

Being an NMDA receptor antagonist, KYNA shares pharmacological properties with phencyclidine and ketamine, substances known to induce psychotic states in healthy individuals, highly resembling those seen in patients.[11,12] Moreover, animals with pharmacologically elevated brain KYNA levels display abnormal behavior consistent with the human schizophrenia phenotype, eg, disrupted prepulse inhibition,[13] memory deficits,[14,15] and decreased set-shifting ability.[16] Accordingly, knockout mice lacking kynurenine transferase II, catalyzing the formation of KYNA from kynurenine, display improved cognitive function.[17]

The kynurenine pathway modulates the dopamine (DA) system, for decades known to be associated with schizophrenia. Thus, chronic elevation of brain KYNA in rodents increases midbrain DA firing,[18] as well as DA release and locomotor activity following a D-amphetamine challenge.[19,20] Conversely, stimulation of DA receptors, tentatively located on astrocytes,[21,22] either indirectly by amphetamine or L-DOPA administration[23,24] or directly by D1 or D2 subtype agonists,[25] decreases rat brain KYNA levels.

Given the neuroactive properties of KYNA, the kynurenine pathway may serve as a link between immune signaling and neuronal activity. Thus, the synthesis of KYNA, critically regulated by TDO and indoleamine 2,3-deoxygenase (IDO), is highly inducible by cytokines.[26,27] In line with this, patients with schizophrenia display, in addition to a facilitated kynurenine pathway of the brain, elevated CSF levels of the proinflammatory cytokines, IL-1β[28] and IL–6.[29] Altogether, these studies suggest that a central immune activation in psychotic disorders may causally lead to activation of the kynurenine pathway.

As both clinical and experimental studies suggest that antipsychotic drugs interact with cytokine signaling,[30–33] we

aimed to investigate the effects of chronic treatment with traditional or atypical antipsychotic drugs on brain cytokines and KYNA.

Materials and Methods

Animals. Twenty-four male Sprague-Dawley albino rats (Charles River, Germany) weighing ~180 g at the start of the experiment, were housed three per cage (12 hours light/dark) with food and water available ad libitum. Four groups were injected i.p. once a day for 30 days with either saline, haloperidol (1.5 mg/kg), clozapine (20 mg/kg), or olanzapine (2 mg/kg). This dosing regimen was tailored to reasonably capture clinical practice, ie, haloperidol, clozapine, and olanzapine with a range of 1–8 mg, 200–450 mg, and 10–20 mg, respectively. At day 31, animals were anesthetized with chloral hydrate (400 mg/kg) and put in a stereotactic apparatus; CSF was collected from the cisterna magna using a 25G needle connected to a Hamilton® syringe, and CSF was quickly frozen. Animals were decapitated, and the brains were removed and put on dry ice and stored at –20 °C until analysis. Experiments were approved by and performed in accordance with the guidelines of the Ethical Committee of Northern Stockholm, Sweden. All efforts were made to minimize the number of animals used and avoid suffering.

Drugs and chemicals. Sterile saline and olanzapine (Zyprexa®) were obtained from Apoteket Farmaci, clozapine from Tocris Bioscience, and haloperidol from Janssen Pharmaceutica. Acetonitrile, EDTA, glucose, sodium acetate, NaOH, $Na_2S_2O_5$, HCl, PBS, and zinc acetate were purchased from Sigma-Aldrich. Chloral hydrate was obtained from Merck.

Analysis of KYNA in brain tissue. Analysis of KYNA was performed essentially as was recently described.[29] Briefly, samples were run isocratically through a reversed-phase 4 × 150 mm ReproSil-Pur C18 column (3 µm; Dr. Maisch GmbH), with a mobile phase running at 0.5 mL/minute (50 mM sodium acetate, 7% acetonitrile in ultrapure dH_2O) by a dual piston HPLC pump (LC-10AD VP, Shimadzu Corporation). A total of 0.5M zinc acetate were delivered postcolumn, and KYNA was analyzed by fluorescence detection (FP-2020 Plus, Jasco Ltd.), with excitation wavelength set to 344 nm, emission wavelength 398 nm. KYNA standard solutions (ranging between 30 and 1 nM) were used as a reference curve. Signals from the fluorescence detector were transferred to a computer for analysis with Datalys Azur® (Grenoble, France).

Cytokine analysis. IL-1β, IL-4, IL-5, IL-8, IL-13, tumor necrosis factor (TNF)-α, and interferon-γ (IFN-γ) were analyzed in CSF from rats using a customized rat Ultra-Sensitive 7-Plex Kit (Meso Scale Discovery). IL-6 was analyzed separately using a customized rat Ultra-Sensitive Kit by the same platform. The assays were analyzed as per the manufacturer's protocol with the modification of a longer primary incubation time (overnight at 4 °C). Intra-assay coefficient of variation was below 20% for all analytes presented. The limits of detection (LODs) in our analyses were IL-1β (32.3 pg/mL), IL-4 (1.14 pg/mL), IL-5 (27.5 pg/mL), IL-8 (2.5 pg/mL), IL-13 (2.84 pg/mL), TNF-α (2.54 pg/mL), IFN-γ (2.09 pg/mL), and IL-6 (39.9 pg/mL). With regard to the cytokine analysis, samples from five rats were excluded from statistical analysis because of failure of collecting CSF, blood contamination of CSF, or values below the detection limits.

Results

IL-1β, IL-4, IL-5, IL-8, IL-13, TNF-α, and IFN-γ in the CSF were analyzed using a Meso Scale Discovery immunoassay 7-plex kit, and IL-6 was analyzed separately also by the Meso Scale platform. Administration of clozapine (20 mg/kg, i.p.) once daily for 30 days was found to markedly reduce CSF IL-8 levels compared to saline treated rats (23.2 ± 2.45 pg/mL vs. 40.1 ± 3.44 pg/mL, $P = 0.037$). In contrast, similar treatments with haloperidol (1.5 mg/kg) or olanzapine (2 mg/kg) did not affect CSF IL-8 levels (34.8 ± 3.51 pg/mL and 34.6 ± 4.80 pg/mL, respectively, Fig. 1). All other cytokines were found to be below the LOD regardless of the chronic antipsychotic treatment. None of the drugs investigated affected basal KYNA levels compared to saline treated controls (Table 1).

Discussion

The results of the present study show that chronic treatment with clozapine, in contrast to that of olanzapine or haloperidol, decreased CSF levels of IL-8, the only detectable CSF cytokine in our experiments. Some clinical and experimental data suggest that the proinflammatory cytokine IL-8 is associated with schizophrenia, but no consistent picture has emerged in this regard. According to a meta-analysis of cytokine alterations in patients with schizophrenia,[34] elevated blood levels of IL-8 is associated with an acute relapse in inpatients. Stratification of patients into responders and nonresponders to antipsychotic medication revealed significantly higher IL-8 in

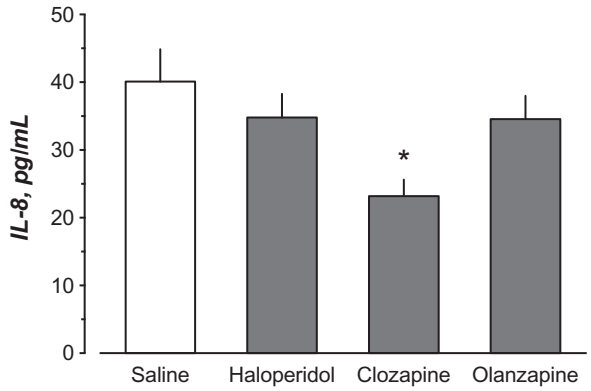

Figure 1. CSF IL-8 in rats treated daily with saline i.p., haloperidol (1.5 mg/kg/day, i.p.; $n = 4$), clozapine (20 mg/kg/day, i.p.; $n = 4$), or olanzapine (2 mg/kg/day, i.p.; $n = 6$) during 30 days. Each bar represents the mean ± SEM. Statistics: $^*P = 0.032$ (Mann–Whitney).

Table 1. KYNA levels in rat whole brain following chronic of treatment with haloperidol, clozapine, or olanzapine.

	N	KYNA (nM) ± SEM
Control (saline)	6	21.33 ± 1.10
Haloperidol (1.5 mg/kg/day)	6	22.70 ± 1.30
Clozapine (20 mg/kg/day)	6	20.02 ± 1.12
Olanzapine (2 mg/kg/day)	6	20.58 ± 0.67

Notes: Values are mean ± SEM. No significant difference between groups was found (Mann–Whitney).

plasma from nonresponders.[35] Also, basal and lipopolysaccharide (LPS)-induced IL-8 production is higher in peripheral blood mononuclear cells (PBMCs) from patients with schizophrenia compared to controls.[36] Notably, elevated maternal plasma levels of IL-8 is associated with neuroanatomical alterations in areas linked to schizophrenia among cases[37] and increases the risk of developing the disease.[38] Our findings are in analogy with a recent study by Chen et al.[39] showing that clozapine, but not haloperidol, reduces IL-8 in human macrophages following an LPS challenge. In human adipocytes, however, clozapine was found to increase mRNA of IL-8 and other cytokines.[40] Notably, our finding that chronic olanzapine treatment failed to affect rat CSF IL-8 levels is coherent with our recent study showing that patients during olanzapine treatment have CSF levels of IL-8 similar to healthy volunteers.[29] The current finding may have clinical relevance as it implies that clozapine has anti-inflammatory properties in the brain and that this may be related to its unique clinical profile, also including increased risk of hematological side effects.[41–44] The mechanism by which clozapine inhibits brain IL-8 release is obscure. Given the relatively similar profile of clozapine and olanzapine in DA- or 5-hydroxytryptamine-receptor binding,[45] it appears unlikely that any of these receptors accounts for the reduction in IL-8. However, clozapine, but not olanzapine, has been shown to act as a partial agonist at the glycine site of the NMDA receptor.[46,47] Given a modulation of IL-8 by the NMDA receptor,[48] this action of clozapine may contribute to the reduction in CSF IL-8 seen after a chronic treatment. Importantly, one should be aware that the action of clozapine on brain immune activation might not be restricted to IL-8 solely. Thus, a major limitation of the present study is that among all cytokines analyzed, IL-8 was the only one that was found above the detection limit.

In the present study, none of the drugs administered affected basal levels of brain KYNA. These findings are in line with previous studies showing the lack of effects on brain KYNA by acute administration of antipsychotic drugs.[13,49] Further, our results are supported by a previous clinical study where drug-naive patients with schizophrenia showed the same magnitude of CSF KYNA elevation as patients on antipsychotics.[6] In a previous experimental study, however, one-month, but not one-week treatment with haloperidol, raclopride, and clozapine, reduced KYNA levels in discrete brain regions of the rat, ie, striatal, hippocampal, and cortical areas.[49] At any rate, present and previous data collectively speak against the possibility that the increased levels of KYNA found in clinical studies are the result of the antipsychotic treatment per se.

Although proinflammatory cytokines are believed to induce the kynurenine pathway by the induction of the rate-limiting enzymes IDO/TDO,[26] the decrease in brain IL-8 seen after the chronic clozapine treatment apparently does not influence the levels of brain KYNA. This principally indicates that this cytokine, in contrast to, eg, IFN-γ and/or IL-1β, is not primarily involved in the modulation of KYNA synthesis.

In summary, the results of the present study show that chronic treatment with clozapine, in contrast to chronic treatment with haloperidol or olanzapine, is associated with decreased rat CSF levels of the proinflammatory cytokine IL-8. Such an effect may participate in the superior clinical efficacy of the drug.

Author Contributions

Conceived and designed the experiments: LS, SE, GE. Analyzed the data: ML. Wrote the first draft of the manuscript: ML. Contributed to the writing of the manuscript: LS, SE, MG, GE. Agree with manuscript results and conclusions: ML, LS, MG, SE, GE. Jointly developed the structure and arguments for the paper: ML, GE. Made critical revisions and approved final version: SE, MG, GE. All authors reviewed and approved of the final manuscript.

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