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Clozapine and olanzapine inhibit proton currents in BV2 microglial cells

Hyewon Shin, Jiwon Kim, Jin-Ho Song *

Department of Pharmacology, College of Medicine, Chung-Ang University, 84 Heukseok-Ro, Dongjak-Gu, Seoul 156-756, Republic of Korea

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ABSTRACT

Excessive reactive oxygen species produced by NADPH oxidase in over-activated microglia can lead to neuronal death. Some atypical antipsychotic drugs possibly have anti-inflammatory properties and suppress the production of pro-inflammatory cytokines and reactive oxygen species from microglia. Voltage-gated proton channels (Hv1) are expressed in microglia and are required for NADPH oxidase-dependent reactive oxygen species generation, which could contribute to neuronal death and ischemic brain damage. In the present study, we examined the effects of the atypical antipsychotics clozapine, olanzapine and risperidone on proton currents in microglial BV2 cells. Clozapine and olanzapine inhibited proton currents with IC_{50} values of 9.8 μM and 84 μM, respectively. Risperidone, however, showed very weak inhibition of proton currents. Clozapine-induced inhibition of proton currents was not accompanied by a positive shift in the activation voltage or reversal potential, indicating that the inhibition was not mediated through an increase in the intracellular pH. Clozapine binds to a multitude of receptors, including serotonin, dopamine and muscarinic receptors. Serotonin receptors, however, were not responsible for the proton current inhibition by clozapine. Of the three drugs, only clozapine could reach concentrations to inhibit microglial proton currents in the brain at therapeutic doses. Thus, the anti-inflammatory activity of clozapine may be partly attributable to its inhibition of microglial proton currents.

1. Introduction

Atypical antipsychotic drugs such as clozapine, olanzapine and risperidone are strong antagonists of serotonin 5-HT_{2A} receptors, as well as dopamine D_2 receptors (Meltzer and Massey, 2011). Although the etiology of schizophrenia remains elusive, neuroinflammation and free radicals may play a role in its pathogenesis (Monji et al., 2013; Reddy and Yao, 1996). In line with this, some antipsychotics possibly have anti-inflammatory properties. For example, clozapine, olanzapine, and risperidone suppress the production of pro-inflammatory cytokines such as tumor necrosis factor-α and interleukin-6 in serum from lipopolysaccharide-treated mice, while clozapine greatly increases the production of the anti-inflammatory cytokine interleukin-10 (Sugino et al., 2009). In drug-naïve patients with first-episode schizophrenia, antioxidant defense is impaired and lipid peroxidation increased, indicative of oxidative injury at the onset of the disease (Mahadik et al., 1998).

The inflammatory response in the central nervous system is dominated by microglia, although peripheral macrophages are also recruited under injurious conditions (Schwartz et al., 2013). Over-activated microglia can cause detrimental neurotoxicity by releasing excess pro-inflammatory cytokines and reactive oxygen species (Block et al., 2007). From this perspective, it is noteworthy that clozapine attenuated lipopolysaccharide-induced microglial activation and reduced the production of superoxide and reactive oxygen species, as well as nitric oxide and tumor necrosis factor-α (Hu et al., 2012). Olanzapine suppressed nitric oxide release from lipopolysaccharide-stimulated microglial cell lines (Hou et al., 2006). Risperidone inhibited the production of nitric oxide and pro-inflammatory cytokines, including interleukin-1β, interleukin-6 and tumor necrosis factor-α from interferon-γ-activated microglial 6–3 cells (Kato et al., 2007).

NADPH oxidase in phagocytes, including microglia, transports electrons across the plasma membrane to generate superoxide and other downstream reactive oxygen species whose main physiological function is host defense (Bedard and Krause, 2007). Pathologically, however, reactive oxygen species generated by microglial NADPH oxidase lead to neuronal death in inflammatory conditions. Moreover, intracellular reactive oxygen species act as second

* Corresponding author. Tel.: +82 2 820 5686; fax: +82 2 817 7115.
E-mail address: jinhos@cau.ac.kr (J.-H. Song).

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messengers to increase pro-inflammatory gene expression in microglia (Block et al., 2007).

Voltage-gated proton channels (Hv1) are predominantly expressed in immune cells and involved in acid extrusion, signaling, and reactive oxygen species generation (DeCoursey, 2013). NADPH oxidase activity results in membrane depolarization and low intracellular pH, effects that are autoinhibitory to NADPH oxidase (DeCoursey, 2013; Morgan et al., 2005). Proton channels sustain NADPH oxidase activity by both dissipating the accumulated acid and compensating for the charge imbalance (El Chemaly et al., 2010; Morgan et al., 2009). Thus, neutrophils from $Hv1^{-/-}$ mice produced less superoxide and reactive oxygen species than neutrophils from wild-type mice (El Chemaly et al., 2010). Since clozapine, olanzapine, and risperidone have been shown to suppress reactive oxygen species production in microglia, we sought to determine whether these atypical antipsychotics could inhibit proton currents in microglial BV2 cells.

2. Materials and methods

2.1. Cells

A mouse microglial BV2 cell line was cultured in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum (WELGENE, Daegu, Republic of Korea), 100 units/mL penicillin, and 100 μg/mL streptomycin (Sigma-Aldrich, St. Louis, MO). Cells were incubated at 36 °C under an atmosphere of 5% CO_2. For experiments, cells were plated onto 12 mm glass coverslips coated with poly-L-lysine in 35 mm culture dishes. These cells were allowed to grow at least two days before being used for electrophysiological recordings.

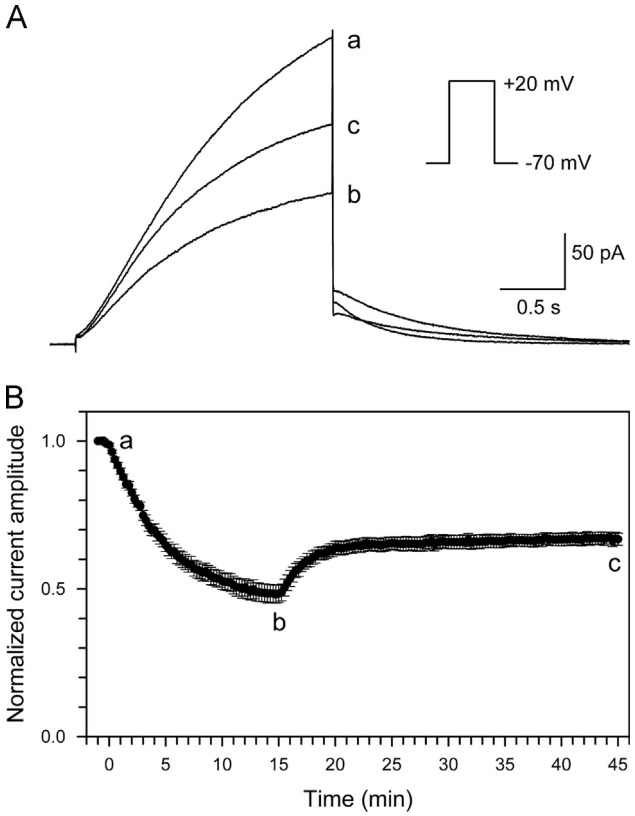

Fig. 1. Clozapine inhibition of voltage-gated proton currents in microglial BV2 cells. Proton currents were induced using 2-s voltage steps to +20 mV from a holding potential of −70 mV every 15 s. The intracellular pH was 5.5 and the extracellular pH was 7.3. (A) Representative current traces. After recording the control current (a), clozapine (10 μM) was applied for 15 min (b), and then removed by perfusion with the external solution for 30 min (c). (B) Time course of proton current inhibition by clozapine ($n=10$). The steady-state current amplitude measured by a fit with an exponential function was normalized to the control current amplitude to produce the plot.

2.2. Solutions

The external solution contained 85 mM *N*-methyl-D-glucamine aspartate, 100 mM HEPES, 1 mM $CaCl_2$, and 1 mM $MgCl_2$. CsOH was used to titrate the pH of the external solution to 7.3. The internal solution contained 120 mM 2-(*N*-morpholino)ethanesulfonic acid (MES), 85 mM *N*-methyl-D-glucamine aspartate, 1 mM EGTA, and 3 mM $MgCl_2$. The pH of the internal solution was titrated to 5.5 using CsOH. Clozapine, olanzapine, risperidone, and serotonin were dissolved in dimethylsulfoxide to obtain 1000-fold stock solutions. The stock solutions were stored at −20 °C in small aliquots and diluted to the desired concentrations in the external solution immediately before experiments. All chemicals were obtained from Sigma-Aldrich.

2.3. Electrophysiological recordings

The whole-cell patch-clamp technique was used to record voltage-gated proton currents. All recordings were performed at room temperature (22–24 °C). Patch pipettes were made of filament-containing borosilicate glass capillaries (G150TF-4, Warner Instrument, Hamden, CT) using a two-step vertical puller (PP83, Narishige, Tokyo, Japan) and the tip was heat-polished with a microforge (MF83, Narishige). Pipette resistance ranged between 6 and 8 MΩ when filled with the internal solution. The reference electrode was an Ag–AgCl wire connected to the bath solution through a 3 M KCl–agar bridge. The liquid junction potential between the internal and external solutions was 1.3 mV and was corrected before gigaohm-seal formation. Currents were recorded with an Axopatch 200B patch-clamp amplifier controlled by pCLAMP 8 software and digitized at 1 kHz with Digidata 1322 A (Molecular Devices, Sunnyvale, CA). Currents were low-pass filtered at 1 kHz. Data are expressed as mean ± S.E.M. and n refers to the number of cells examined. Student's t-test was used for comparisons, and statistical significance was set at $P<0.05$.

3. Results

3.1. Clozapine inhibits voltage-gated proton currents

Voltage-gated proton currents in microglial BV2 cells in response to 2-s depolarizing steps to +20 mV from a holding potential of −70 mV at an intracellular/extracellular pH gradient of 5.5/7.3 are shown in Fig. 1. The outward current, with slow activation kinetics and no clear inactivation during the 2-s depolarization, is characteristic of a proton current. Clozapine inhibited this proton current. The steady-state current amplitude was measured by a fit with an exponential function. In the presence of clozapine (10 μM), the current amplitude was gradually reduced, reaching an apparent steady-state in 15 min at 48 ± 3% of the control current ($n=10$). Upon washout with the external solution for 30 min, the current recovered only partially to 67 ± 4% of the control current.

3.2. Concentration–response relationship

The inhibition of proton currents by clozapine was measured at different concentrations and a concentration–response relationship curve was calculated (Fig. 2). The current-amplitude data