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Research Report

Temporal alteration of spreading depression by the glycine transporter type-1 inhibitors NFPS and Org-24461 in chicken retina

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ABSTRACT

We used isolated chicken retina to induce spreading depression by the glutamate receptor agonist N-methyl-D-aspartate. The N-methyl-D-aspartate-induced latency time of spreading depression was extended by the glycine$_B$ binding site competitive antagonist 7-chlorokynurenic acid. Addition of the glycine transporter type-1 inhibitors NFPS and Org-24461 reversed the inhibitory effect of 7-chlorokynurenic acid on N-methyl-D-aspartate-evoked spreading depression. The glycine uptake inhibitory activity of Org-24461, NFPS, and some newly synthesized analogs of NFPS was determined in CHO cells stably expressing human glycine transporter type-1b isoform. Compounds, which failed to inhibit glycine transporter type-1, also did not have effect on retinal spreading depression. These experiments indicate that the spreading depression model in chicken retina is a useful *in vitro* test to determine activity of glycine transporter type-1 inhibitors. In addition, our data serve further evidence for the role of glycine transporter type-1 in retinal neurotransmission and light processing.

1. Introduction

Spreading depression, a slowly propagating wave of depression of electrical activity, is a phenomenon which occurs in pathological conditions such as stroke and migraine aura (Dohmen et al., 2008; Ayata, 2010). In the retina, spreading depression is a visible light scattering (Martins-Ferreira and Oliveira-Castro, 1966) that is mediated by the release of the excitatory neurotransmitter glutamate (Sheardown, 1993). It has been shown that drugs either inhibiting or potentiating the glutamatergic system have influence on retinal spreading depression (Kapus et al., 2004; Kertesz et al., 2010; Wang et al., 2012). Of the ionotropic glutamate receptor agonists, N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate induce spreading depression in the chicken retina and the latter compounds evoke this change via indirect activation of NMDA receptors (Sheardown, 1993). NMDA receptors appear to be rare or absent in the outer retina and

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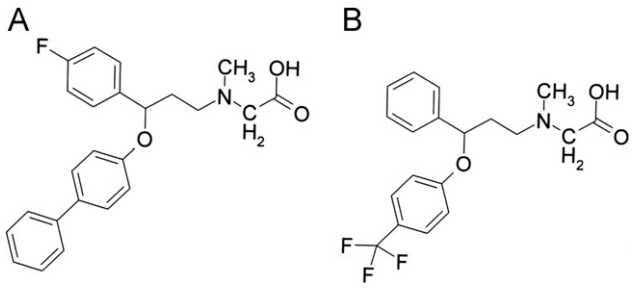

Fig. 1 – Chemical structures of GlyT-1 inhibitors: sarcosine derivatives used in this study. (A) NFPS (N-[3-(4′-fluorophenyl)-3-(4′phenylphenoxy)propyl]sarcosine) and (B) Org-24461 (N-methyl-N-[(4-trifluoromethyl)phenoxy]-3-phenyl-propyl-glycine).

NMDA receptor antagonists have little or no effect on the bipolar second-order neurons (Linn and Massey, 1991). These receptors in the retina are mainly concentrated on amacrine cells and ganglion cells in the inner plexiform and nuclear layers and the ganglion cell layer (Fletcher et al., 2000). NMDA receptors enhance the light-evoked responses of retinal ganglion cells whereas those located on amacrine cells could enhance feed forward inhibition to ganglion cells (Reed et al., 2009). However, NMDA receptor activation requires the presence of glycine, a coagonist of NMDA receptors (Johnson and Ascher, 1987). Concentrations of glycine in glutamatergic synapses and around NMDA receptors are regulated by the glycine transporter type-1 (GlyT-1) (Bergeron et al., 1998; Musante et al., 2011). Studies have demonstrated that NMDA receptors and GlyT-1 are found in close vicinity in the CNS (Cubelos et al., 2005) and these receptors and GlyT-1 may be in a functional link in the retina as well. The presence and the distribution of GlyT-1 in the retina were demonstrated by using immunocytochemical techniques and it was found that GlyT-1 is expressed in most glycinergic amacrine cells (Zafra et al., 1995; Wässle et al., 2009; Harsing et al., 2012a). Functional studies carried out in GlyT-1-deficient mouse demonstrated that GlyT-1 in the retina determines the saturation of glycine$_B$ binding site at NMDA receptors (Reed et al., 2009).

Since spreading depression can be reproducibly evoked in the retinas of 3 to 7-day old chicken this model has been frequently used to determine the activity of the 2,3-benzodiazepine AMPA receptor antagonists (Abraham et al., 2000; Kertesz et al., 2004), the NMDA receptor ion-channel blocker MK-801 or memantine (Kertesz et al., 2010). We report here that the glycine$_B$ binding site competitive antagonist 7-chlorokynurenic acid (Danysz and Parson, 1998) extends the latency of NMDA-induced spreading depression in young chicken retinas and this effect can be reversed by the GlyT-1 inhibitors sarcosine, and the sarcosine derivatives NFPS, and Org-24461 (Fig. 1).

2. Results

2.1. *Effects of NMDA receptor ligands on spreading depression*

The NMDA-induced latency time of spreading depression in isolated chicken retina was about 50 s when 35 μM NMDA was used. We experienced that spreading depression could hardly be elicited by NMDA at concentrations lower than 30 μM (at 1 mM Mg^{2+} concentration in the buffer), probably because of the insufficient removal of Mg^{2+} block. The latency time of spreading depression was decreased to 15–20 s as the applied concentration of NMDA was increased to 100 μM. We could reliably induce spreading depression at this concentration of NMDA and the effect of NMDA could be modified consistently with the NMDA receptor antagonist 7-chlorokynurenic acid.

The NMDA (100 μM)-induced latency time of spreading depression was extended to about 1 min by addition of 3 μM of 7-chlorokynurenic acid. In our preliminary study, higher concentrations (≥5 μM) of 7-chlorokynurenic acid inhibited spreading depression with smaller variability in delay but also with smaller reversibility. Lower concentrations of 7-chlorokynurenic acid inhibited spreading depression less consistently. Therefore, 3 μM 7-chlorokynurenic acid was considered to cause consistent and reversible inhibition of NMDA-evoked spreading depression, which then was taken 100%. 7-Chlorokynurenic acid (0.3–3 μM) concentration dependently extended the spreading depression latency time induced by 100 μM NMDA (Fig. 2A). Glycine added in a concentration of 100 μM to chicken retinas reduced the NMDA-induced latency time of spreading depression in the presence of 7-chlorokynurenic acid by 25–50 %.

2.2. *Effects of GlyT-1 inhibitors on glycine uptake and spreading depression*

Sarcosine (0.1–1 mM) reduced the extension effect of 7-chlorokynurenic acid on NMDA-induced spreading depression latency time and its calculated IC_{50} value was 247 μM (Fig. 2B). NFPS and Org-24461 added in concentrations of 0.1–1 μM also reversed the inhibitory effect of 7-chlorokynurenic acid on NMDA-induced latency time (Fig. 2B). The calculated IC_{50} values of NFPS and Org-24461 for reversal of the inhibitory effect of 7-chlorokynurenic acid on NMDA-induced latency time were 0.31 and 0.36 μM, respectively (Table 1). For comparison, the IC_{50} values of NFPS and Org-24461 to inhibit hGlyT-1b permanently expressed in CHO cells were 40 and 100 nM, respectively (Table 1). NFPS and Org-24461 also decreased NMDA-induced latency time of spreading depression in the absence of 7-chlorokynurenic acid but their concentrations required were found much higher and fall in the 10–30 μM concentration range. When added by themselves, neither NFPS (10 μM) nor Org-24461 (30 μM) induced spreading depression in isolated chicken retina. The GlyT-1 inhibitors tested here also reduced the AMPA (2 μM)-induced latency of spreading depression in chicken retinas, their required concentrations for this effect were again in the range of 10–30 μM. Analogs of NFPS (SzV-186, SzV-220, and SzV-221), which did not exhibit marked GlyT-1 inhibitory effects (IC_{50} values to inhibit hGlyT-1b permanently expressed in CHO cells were higher than 10 μM), were ineffective in reversal of 7-chlorokynurenic acid-induced extension of NMDA-induced spreading depression (Table 1). Clozapine (1–100 μM), an antipsychotic with weak GlyT-1 inhibitory activity (Williams et al., 2004), was also found ineffective in this test (data not shown).