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Zinc reduces antiseizure activity of neurosteroids by selective blockade of extrasynaptic GABA-A receptor-mediated tonic inhibition in the hippocampus

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Abstract

Zinc is an abundant trace metal in the hippocampus nerve terminals. Previous studies demonstrate the ability of zinc to selectively block neurosteroid-sensitive, extrasynaptic GABA-A receptors in the hippocampus (Carver et al, 2016). Here we report that zinc prevents the seizure protective effects of the synthetic neurosteroid ganaxolone (GX) in an experimental model of epilepsy. GABA-gated and tonic currents were recorded from dissociated dentate gyrus granule cells (DGGCs), CA1 pyramidal cells (CA1PCs), and hippocampal slices from adult mice. Antiseizure effects of GX and the reversal of these effects by zinc were evaluated in fully-kindled mice expressing generalized (stage 5) seizures. In electrophysiological studies, zinc blocked the GABA-evoked and GX-potentiated GABA-gated chloride currents in DGGCs and CA1PCs in a concentration-dependent fashion similar to the competitive GABA-A receptor antagonists bicuculline and gabazine. Zinc completely blocked GX potentiation of extrasynaptic tonic currents, but not synaptic phasic currents. In hippocampus kindling studies, systemic administration of GX produced a dose-dependent suppression of behavioral and electrographic seizures in fully-kindled mice with complete seizure protection at the 10 mg/kg dose. However, the antiseizure effect of GX was significantly prevented by intrahippocampal administration of zinc (ED_{50}, 150 μM). The zinc antagonistic response was reversible as animals responded normally to GX administration 24 h post-zinc blockade. These results demonstrate that zinc reduces the antiseizure effects of GX by selectively blocking extrasynaptic δGABA-A receptors in the hippocampus. These pharmacodynamic interactions have clinical implications in neurosteroid therapy for brain conditions associated with zinc fluctuations.

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Conflict of Interest

None

Keywords

Zinc; ganaxolone; GABA-A receptors; tonic inhibition; hippocampus; seizures

1. Introduction

The hippocampus is a key region associated with the pathophysiology of epilepsy (Jutila et al., 2002). There are two distinct categories of GABA-A receptors in the hippocampus. Synaptic receptors and extrasynaptic receptors exhibit different characteristics in their affinity and efficacy to GABA, desensitization rate, and drug sensitivity (Bianchi and Macdonald, 2002; 2003; Brown et al., 2002; Mortensen et al., 2011; Wohlfarth et al., 2002). Synaptic receptors, mainly γ- containing receptors, produce rapid and transient phasic currents in response to presynaptic release of GABA (~1 mM), whereas extrasynaptic receptors, mostly δ-containing receptors in dentate gyrus and α5β3γ2 receptors in CA1, generate persistent, non-desensitizing tonic currents by ambient GABA (~0.3−2 μM) (Mody et al., 1994; Brickley et al., 1996; Farrant and Nusser, 2005). Tonic currents contribute to the overall basal tone and shunting inhibition via continuous channel conductance in neurons expressing δ-containing receptors. Hippocampus granule cells (DGGCs), which express high density of δ-subunit-containing extrasynaptic receptors, promote tonic inhibition and thereby regulate network excitability (Coulter and Carlson, 2007; Glykys et al., 2008). GABA-A receptors are modulated by many compounds including benzodiazepines, neurosteroids, and zinc (Zn^{2+}).

Zn^{2+} is the second most abundant trace metal in many cells and neurons in the brain. It regulates cellular functions such as gene expression, epigenetic enzymatic activity, protein structural stability, and neuronal plasticity. Zn^{2+} is particularly abundant in the cortex and limbic system, including hippocampus and amygdala (Frederickson, 1989; Frederickson et al., 2000; 2005; Gower-Winter and Levenson, 2012; Szewczyk, 2013). Zn^{2+} homeostasis in the brain is tightly controlled and primarily regulated by Zn^{2+} transporters and binding proteins. High level of “chelatable” Zn^{2+} is sequestered in the synaptic vesicles of glutamatergic neurons through Zn^{2+} transporter protein ZnT3. Other transporters like ZIP proteins promote the efflux of vesicular Zn^{2+} and increase the concentration of cytoplasmic Zn^{2+} (Cole et al., 1999; Kambe et al., 2004; Lee et al., 2011). Zn^{2+} is synaptically co-released with glutamate from presynaptic vesicles of the hippocampal mossy fibers, the axons of DGGCs (Assaf and Chung, 1984; Frederickson et al., 1983; Molnar and Nadler, 2001; Paoletti et al., 2009). Zn^{2+} has been shown to modulate both excitatory and inhibitory transmission (Westbrook and Mayer, 1987; Harrison and Gibbons, 1994; Vogt et al., 2000). Zn^{2+} inhibits GABA-A receptors and has distinct sensitivities at different receptor subtypes partly due to the accessibility of Zn^{2+} binding sites (Smart et al., 1991; Barberis et al., 2000; Ruiz et al., 2004). The δ-containing extrasynaptic receptors exhibit greater sensitivity to Zn^{2+} blockade than γ-containing synaptic receptors (Hosie et al., 2003). Alterations in Zn^{2+} homeostasis have implications in several neurological disorders (Frederickson et al., 2005; Bitanihirwe and Cunningham, 2009; Qian et al., 2011; Szewczyk, 2013; Prakash et al., 2015). Aberrant Zn^{2+}-rich mossy fiber sprouting is a classical feature in limbic epileptogenesis (Cavazos et al., 1991). Excessive release of Zn^{2+} drastically alters the

excitability of hippocampal circuits and plays an important role in the pathophysiology of epilepsy (Takeda et al., 1999; Coulter, 2000; Foresti et al., 2008; Elsas et al., 2009).

Allopregnanolone (3α-hydroxy-5α-pregnan-20-one, AP) and related neurosteroids are endogenous modulators of neuronal excitability and seizure susceptibility (Chuang and Reddy, 2018; Younus and Reddy, 2018). Neurosteroids modulate GABA-A receptors primarily via allosteric potentiation of GABA at nanomolar concentrations and through direct activation of the channel at micromolar concentrations (Hosie et al., 2007). AP has powerful antiseizure activity (Carver et al., 2016; Kaminski et al., 2003; Kaminski et al., 2004; Kokate et al., 1996; Reddy and Estes, 2016). Ganaxolone (3α-hydroxy-3β-methyl-5α-pregnan-20-one, GX), a synthetic analog of AP, is designed to treat epilepsy and related seizure conditions. GX produces robust positive allosteric modulation of GABA-A receptors, with preferential sensitivity at δ-subunit-containing extrasynaptic receptors (Chuang and Reddy, 2018b). GX has a more favorable biopharmaceutical profile than AP due to its higher bioavailability and less hormonal side effects (Carter et al., 1997; Reddy and Woodward, 2004; (Carver and Reddy, 2016). GX produces powerful antiseizure activity in a wide range of experimental models, and is being evaluated in clinical trials for epilepsy (Bialer et al., 2015; Braat et al., 2015; Kerrigan et al., 2000; Laxer et al., 2000; Nohria and Giller, 2007; Pieribone et al., 2007; Reddy and Rogawski, 2012; Sperling et al., 2017). Recently, Zn^{2+} has been shown to selectively block neurosteroid-sensitive, extrasynaptic δGABA-A receptors in the hippocampus (Carver et al, 2016). Therefore, it is likely that Zn^{2+} may exert antagonistic effects on GX modulation of tonic inhibition and seizure protection.

In the present study, we investigated the pharmacodynamic interactions of Zn^{2+} and GX at extrasynaptic GABA-A receptors and its functional relevance on the anticonvulsant activity of GX in the kindling model. Our results demonstrate that Zn^{2+} diminishes the antiseizure effects of GX by selectively blocking the extrasynaptic δGABA-A receptors in the hippocampus.

2. Materials and methods

2.1. Animals

Young adult (2–3 months-old) male mice of C57BL/6–129SV background strain were used in this study. Animals were group housed in an environmentally controlled facility with a 12 h light/dark cycle. The animals were cared for in compliance with the guidelines in the National Institutes of Health *Guide for the Care and Use of Laboratory Animals.* All animal procedures were performed in a protocol approved by the university's Institutional Animal Care and Use Committee.

2.2. Hippocampal slice preparation

Coronal slices (320 μm thickness) of hippocampus were prepared from mice using standard techniques (Carver et al., 2016). Mice were anesthetized with isoflurane and brains were excised rapidly and placed in 3.5˚C artificial cerebrospinal fluid (aCSF) buffer that composed of (in mM): 126 NaCl, 3 KCl, 0.5 $CaCl_2$, 5 $MgCl_2$, 26 $NaHCO_3$, 1.25 NaH_2PO_4,

11 glucose, and 0.3 mM kynurenic acid (pH adjusted to 7.35–7.40 with 95% O_2 - 5% CO_2, 305–315 mOsm/kg). Hippocampal slices were cut with a Vibratome in 3.5˚C aCSF (model 1500 with 900 Refrigeration System; Leica Microsystems, Inc., Bannockburn, IL). For each electrophysiology experiment, 2–4 animals were used for each group and concentration tested.

2.3. Dissociation of neurons

Acute neuron dissociation was performed by an enzymatic technique as described previously (Kay and Wong, 1986; Reddy and Jian, 2010). The hippocampal pieces of the DG and CA1 regions were microdissected carefully under a microscope (model SMZ 647; Nikon, Tokyo, Japan) and incubated in aCSF for 1 h at 28°C. The isolated sections were transferred into an enzymatic solution consisting of aCSF with protease XXIII (3 mg/ml, Sigma-Aldrich, St. Louis, MO). The slices were then incubated for precisely 23 to 25 min at 28°C. The remaining slices were rinsed twice with aCSF and gently triturated through Pasteur pipettes with three distinct diameters (fire-polished) to yield single cells. For each batch, slices were triturated six to nine times with each pipette in approximately 1.5 ml of aCSF. Trituration is proceeded from largest diameter to smallest diameter pipette. Then, the solution with tissue was allowed to settle for 1 min, and the suspension of freshly dispersed cells was carefully plated onto the recording chamber (Warner Instruments, Hamden, CT) for electrophysiology experiments.

2.4. Recording of GABA-gated currents

Whole-cell patch-clamp configuration was used for electrophysiological recordings in dissociated cells (Reddy and Jian, 2010). Experiments were performed at room temperature (22–24 °C). The recording chamber was fixed into the stage of an inverted microscope with phase-contrast and differential interference contrast optics (model IX71; Olympus, Tokyo, Japan). The physiological bath solution for the recording of dissociated neurons was composed of (in mM): 140 NaCl, 3 KCl, 10 HEPES, 2 $MgCl_2$, 2 $CaCl_2$, and 16 glucose (pH adjusted to 7.4 with NaOH, osmolarity, 315–325 mOsm/kg). Cells were visualized and images were acquired through video camera CCD-100 (Dage-MTI, Michigan City, IN) with FlashBus Spectrim 1.2 software (Pelco, Clovis, CA). Recording pipettes were pulled from capillary glass tubes (King Precision Glass, Claremont, CA) using a P-97 Flaming-Brown horizontal puller (Sutter Instrument Company, Novato, CA). The pipette tip resistances were 4 to 6 MΩ for recording. The recording pipettes were filled with a cesium pipette solution containing (in mM): 124 CsCl, 20 tetraethylammonium, 2 $MgCl_2$, 10 EGTA, 10 HEPES, 0.1 GTP, 4 ATP (pH adjust to 7.2 with CsOH, osmolarity, 295–305 mOsm/kg). Currents were recorded using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA). The membrane capacitance, series resistance, and input resistance of the recordings were monitored by applying a 5-mV (100-ms) depolarizing voltage step from a holding potential of −70 mV for dissociated cells. Signals were low-pass filtered at 2 kHz and digitized at 10 kHz with Digidata 1440A system. The current values were normalized to cell capacitance (an index of cell size) and expressed as current density (pA/pF). For whole cell current from isolated single cells, current response was expressed as the percentage of potentiation produced by GABA or GABA+GX. For fast application of test drugs, the perfusion pipette was positioned <200 μm away from the cell in the chamber. GABA, GX, and Zn^{2+} (1–30

μM) were applied using a multi-channel perfusion system (Automate Scientific, Berkeley, CA). A two-minute wash with bath solution was instituted after each drug trial in order to prevent receptor desensitization.

2.5. Slice patch-clamp electrophysiology

Electrophysiological recordings in hippocampal slices were performed in the whole-cell patch- clamp configuration (Carver et al., 2014; Wu et al., 2013). Hippocampal neurons were visually identified with an Olympus BX51 microscope equipped with a 40x water-immersion objective, infrared-differential interference contrast optics, and video camera. Hippocampal slices (320 μm) were maintained in continuously oxygenated aCSF at 28°C in a holding chamber for 60 min, and experiments were performed at room temperature (22–24 °C). The physiological bath solution for whole-cell recording from slice were composed of (in mM): 124 NaCl, 3 KCl, 1.5 $MgSO_4$, 2.4 $CaCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$ and 10 glucose (pH adjusted to 7.4 with NaOH, osmolarity, 295–305 mOsm/kg). The pipette tip resistances were 5 to 7 MΩ for slice recordings, and were filled with a cesium pipette solution containing (in mM): 124 CsCl, 20 tetraethylammonium-chloride, 2 $MgCl_2$, 10 EGTA, 10 HEPES, 0.1 GTP, 4 ATP, and 5 lidocaine *N*-ethyl bromide (QX-314), (pH adjusted to 7.2 with CsOH, osmolarity, 295–305 mOsm/kg). Currents were recorded using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA). The membrane capacitance, series resistance, and input resistance of the recordings were monitored by applying a 5-mV (100-ms) depolarizing voltage step from a holding potential of −65 mV for slice recordings. Signals were low-pass filtered at 2 kHz and digitized at 10 kHz with Digidata 1440A system.

2.6. Tonic current recording and analysis

Tonic current and phasic, miniature inhibitory postsynaptic currents (mIPSCs) of GABA-A receptors were recorded in the presence of tetrodotoxin (TTX, 0.5 μM, Na^+ channel blocker and inhibition of action potential-evoked neurotransmitter release), D,L-2-amino-5-phosphonovaleric acid (APV, 40 μM, N-methyl-D-aspartate channel blocker), and 6,7-dinitroquinoxaline-2,3-dione (DNQX, 10 μM, non-N-methyl-D-aspartate glutamate receptor blocker). The competitive antagonist gabazine (SR-95531, 50 μM) was added to perfusion at the conclusion of slice recordings to confirm block of GABAergic currents. Drugs were delivered to the bath chamber using a multi-channel perfusion system (Automate Scientific, Berkeley, CA). Perfusion rate was maintained at 2 ml/min. Drugs were allowed 2–4 min to perfuse into the bath chamber and slice before measurements were obtained. Off-line current analysis was performed with pClamp 10.2 software (Molecular Devices, Sunnyvale CA) and in-house software. To study the tonic inhibitory currents, transient events were manually removed from the current trace, so that the recording consisted only of the holding current in the voltage-clamp mode (Wu et al., 2013). Averaged amplitude of tonic current shift in conductance and root-mean-square (RMS) noise amplitude were measured. The tonic current was expressed as the difference in holding current before and after application of gabazine (50 μM) or Zn^{2+} (0.1–10 mM). I_{RMS} is the noise conductance from chloride ions passing through the opened channels and in proportion to the chloride driving force. Tonic current was measured and averaged in 100 ms per epoch with 1 sec interval between epochs for 30 epochs. The measurements were taken 30 s before and 2–3 min after application of a

drug. I_{RMS} was studied in 50 ms each epoch with 500 ms interval between epochs for 30 epochs before and after drug application in each cell. To assess the effect of a drug on I_{RMS} in an individual neuron, the distribution of I_{RMS} in 30 epochs before the application of a drug (during the baseline period) was compared with that after drug application by a Student's independent t-test. To compare data obtained from a group of neurons, I_{RMS} values in individual epochs before and after drug application were averaged. Changes in I_{RMS} are expressed in pA of current. Currents for a single cell were normalized to membrane capacitance (pA/pF) as tonic current density. Synaptic currents were recorded and analyzed as previously described (Carver et al., 2016). mIPSCs were acquired for 3 min for each drug response and condition. The amplitude and kinetics of mIPSCs were measured using MiniAnalysis software (Synaptosoft). Nonoverlapping events with single peaks were used to create an ensemble average mIPSC. A mean weighted decay time constant was determined from biexponential fitting function $I(t) = A_1 \times e^{(-t/\tau 1)} + A_2 \times e^{(-t/\tau 2)}$ as $\tau_w = (A_1 \times \tau_1 + A_2 \times \tau_2)/(A_1 + A_2)$. For each electrophysiology experiment, 2–4 animals were used per group.

2.7. Zinc administration

Zinc doses were given acutely via intrahippocampal microinfusion in mice, as previously described (Carver et al., 2016). The sterile saline was used as a control. Apart from a bipolar electrode in the hippocampus, a 26 gauge guide cannula (Plastics One) was stereotaxically implanted into the left ventral hippocampus (2.9 mm posterior, 3.0 mm lateral, and 3.0 mm below dura). After a period of at least 1 week of recovery, dual-implanted animals were subjected to kindling. Zinc was dissolved in sterile saline. Mice received zinc doses (10–300 μM) by slow intrahippocampal microinfusion 10 min prior to GX administration and 25 min before kindling stimulations. The efficiency of zinc delivery was confirmed by the correct placement of the guide cannula by histology (Carver et al., 2016).

2.8. Hippocampus kindling model of epilepsy

A hippocampus kindling model was used in seizure experiments (Reddy and Mohan, 2011; Reddy et al., 2015). Mice were anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). A bipolar electrode fixed to a guide cannula (Plastics One) was stereotaxically implanted in the right ventral hippocampus (2.9 mm posterior, 3.0 mm lateral, and 3.0 mm below dura). After postoperative recovery, animals were subjected to kindling stimulation (Reddy and Mohan, 2011). The electrographic afterdischarge (AD) threshold was determined by application of 1 ms biphasic rectangular pulses at 60 Hz for 1 s, in increments of 25 μA using an isolated pulse stimulator (A-M Systems). AD duration was the total duration of electrographic spike activity (amplitude >2X baseline) occurring in a rhythmic pattern at a frequency >1 Hz. Mice were stimulated at 125% AD threshold once per day until Stage 5 seizures were elicited on 3 consecutive days, considered the fully kindled state. The electrographic activity was recorded using Axoscope 8.0 software with Digidata 1322A interface (Molecular Devices) through a Grass CP511 preamplifier (Astro-Med). Behavioral seizures were rated according to Racine's scale as modified for mouse (Racine, 1972). One week after kindling, $ZnCl_2$ (10 –300 μM) was dissolved in sterile saline and microinfused in 5 μl volume directly into the hippocampus using a perfusion pump at

0.2 μl/min. Mice were monitored for seizures and electrographic activity for at least 10 min. GX (s.c.) was administered 15 min after infusion of Zn^{2+}. GX was dissolved in 20% β-cyclodextrin solution for subcutaneous injections. The control group received this vehicle 15 min prior to kindling stimulations. Mice were scored for protection based on the behavioral motor seizures and AD duration after each kindling session.

2.9. Drugs and reagents

All chemicals used in electrophysiology studies were purchased from Sigma-Aldrich unless otherwise specified. Ganaxolone was prepared as 2 mM stock solutions in dimethyl sulfoxide for electrophysiology experiments. Stock solutions were diluted in the external perfusion solution to the desired concentration for electrophysiological use. The concentration of dimethyl sulfoxide in final solution was less than 1%. GX and kynurenic acid were acquired from Tocris. TTX was purchased from Calbiochem (Billerica, MA).

2.10. Statistical analysis

Data was expressed as the mean ± standard error of mean (SEM). In electrophysiology studies, concentration-response curve data were subjected to non-linear, logistic fitting. Statistical comparisons of parametric measures including electrophysiology data were performed using one-way analysis of variance followed by *post hoc* Tukey's test. In kindling epilepsy studies, differences in seizure stages and AD duration between groups were compared with the nonparametric Kruskal-Wallis test followed by the Mann-Whitney U-test. For patch-clamp data with small sample size, nonparametric tests were used to check the statistical significance especially when the data points were not normally distributed. In all statistical tests, the criterion for statistical significance was $p<0.05$, unless otherwise specified.

3. Results

3.1. Concentration-dependent inhibition of GX potentiation of GABA-gated currents by Zn^{2+} in hippocampal neurons

We first examined the blockade of GABAergic chloride currents with Zn^{2+}, a negative allosteric modulator of GABA-A receptors (Barberis et al., 2000; Carver et al., 2016; Kapur and Macdonald, 1997; Smart et al., 1991). To examine the effects of subunit composition on Zn^{2+} inhibition of GABA-A receptor, concentration-response profiles were compiled in two cell types: δ-abundant DGGCs and δ-sparse CA1 pyramidal cells (CA1PCs). 3 μM GABA (EC_{10}) was applied for 5 seconds followed by the application of Zn^{2+} for 5 seconds (Fig. 1A−C). GABA at 3 μM induced 249.5 ± 16.5 and 538.1 ± 34.3 pA of currents in DGGCs and CA1PCs, respectively. Zn^{2+} blocked inhibitory GABAergic chloride currents concentration-dependently in both cell types. The Zn^{2+} at 1, 3, 10, and 30 μM application blocked a mean 56.7 ± 6.9 %, 81.6 ± 6.4 %, 89.0 ± 5.7 %, and 90.4 ± 5.6 % of GABA-induced currents in DGGCs, respectively. While the Zn^{2+} at 1, 3, 10, and 30 μM blocked a mean 64.3 ± 11.4 %, 70.4 ± 12.0 %, 87.3 ± 3.6 %, and 93.1 ± 1.6 % of GABA-induced currents in γ2-containing CA1PCs, respectively. GABA- modulated currents fully returned to peak amplitude after the removal of Zn^{2+} application. The half maximal inhibitory concentration (IC_{50}) for Zn^{2+} block of 3 μM GABA-induced currents were 0.72 and 0.70

μM in DGGCs and CA1PCs, respectively. These results showed a similar concentration-dependent profile of Zn^{2+} blockade of GABA-gated chloride currents in DGGCs and CA1PCs.

Next, we studied the modulatory effects of Zn^{2+} on GX-potentiated GABA-gated currents. GX at 1 μM was co-applied with 3 μM GABA to obtain the fractional potentiation of GABAergic currents mediated by GX. Subsequent application of Zn^{2+} (1–30 μM) blocked whole-cell inhibitory currents in a concentration-dependent manner (Fig. 2A−C). In δ-containing DGGCs, the IC_{50} for Zn^{2+} block of 3 μM GABA + 1 μM GX currents was 0.44 μM. The 1 μM Zn^{2+} application blocked a mean 62.2 ± 11.6 % of whole-cell current modulation by GX. The 3 μM Zn^{2+} application blocked a mean 77.4 ± 10.5 % of whole-cell current modulation by GX. The 10 μM Zn^{2+} application blocked a mean 86.1 ± 4.8 % of whole-cell current modulation by GX. A 30 μM concentration of Zn^{2+} completely blocked GABA-A receptor whole-cell currents, similar to that of the competitive antagonism of channels by bicuculline and gabazine. In γ-containing CA1PCs, the sensitivity of Zn^{2+} inhibition on GX-induced currents was modestly reduced compared to that of Zn^{2+} in DGGCs (Fig. 2B). The IC_{50} for Zn^{2+} block of 3 μM GABA + 1 μM GX currents was 0.93 μM, which is 2.1-fold higher than that of Zn^{2+} in DGGCs (0.44 μM). The 1 μM Zn^{2+} application blocked a mean 48.7 ± 10.7 % of whole-cell current modulation by GX. The 3 μM Zn^{2+} application blocked a mean 58.1 ± 11.9 % of whole-cell current modulation by GX. The 10 μM Zn^{2+} application blocked a mean 84.5 ± 8.7 % of whole-cell current modulation by GX. A 30 μM concentration of Zn^{2+} blocked a mean 87.3 ± 8.2 % of whole-cell currents. Overall, our results demonstrated that concentration-dependent blockade of Zn^{2+} on GX-potentiated GABA- gated chloride currents has higher sensitivity at neurons that have a higher expression of δ- containing GABA-A receptors.

3.2. Selective antagonism of GX potentiation of tonic currents by Zn^{2+} in hippocampal slices

To examine the interactions of Zn^{2+} with GX at extrasynaptic δ-containing GABA-A receptors, we recorded GX-activated tonic currents from DGGCs in the hippocampus slice using whole-cell voltage-clamp (−65 mV) recording. At the end of each recording, 50 μM gabazine was perfused to determine the total tonic current shift. Tonic current of each cell was normalized to the cell capacitance as a measure of current density (pA/pF). A physiological concentration of GABA (0.2 μM) was used to examine GX allosteric potentiation (Wlodarczyk et al., 2013). Our results showed that application of 0.2 μM GABA + 0.3 μM GX resulted in a negative shift in the holding current level and an increase in the RMS channel conductance (Fig. 5A). However, Zn^{2+} (100 μM) perfusion positively shifted GX-potentiated tonic currents (current density: GABA + GX, 0.96 ± 0.18 pA/pF; GABA + GX + Zn^{2+}, 0.72 ± 0.11 pA/pF, n = 5 cells). To further demonstrate the pharmacological sensitivity of Zn^{2+} blockade of GX-induced tonic current potentiation, 1 μM of GABA and GX were used to record tonic responses (Fig. 3A). Subsequent application of Zn^{2+} (0.1–10 mM) induced a concentration-dependent blockade of tonic current density, measured as positive shift of the holding currents (Fig. 3B). Zn^{2+} (100 μM) wash-out reversed the holding currents to the previously enhanced level by GX. However, with the application of 1 mM Zn^{2+}, Zn^{2+} wash-out was not able to eliminate the effects induced by Zn^{2+}. The IC_{50}

value for Zn^{2+} blockade of tonic currents was 0.186 mM (Fig. 3C). The mean and the standard deviation (s.d.) of the tonic currents for different Zn^{2+} concentrations were plotted in Fig. 3D. Higher the mean of tonic currents was accompanied with higher the s.d. of tonic currents. Zn^{2+} concentration- dependently reduced both the mean and s.d. of GX-potentiated tonic currents. Zn^{2+} at 0.1, 1, and 10 mM caused 56.2 ± 6.0 %, 60.8 ± 3.0 %, and 98.3 ± 0.7 % reduction of GX-potentiated tonic currents, respectively. Zn^{2+} also significantly reduced GX-potentiated RMS channel conductance at all concentration tested (Fig. 3E). Zn^{2+} at 10 mM caused 42.0 ± 1.7 % reduction of the RMS channel conductance, similar to that of the competitive antagonist gabazine (44.0 ± 1.8 %). Overall, these results demonstrate a concentration-dependent blockade of Zn^{2+} on GX- potentiated tonic currents.

3.3. GX potentiation of phasic currents are insensitive to Zn^{2+} antagonism

Next, to examine the block of Zn^{2+} on GX-potentiated phasic currents in DGGCs, we recorded GX-activated mIPSCs, which primarily reflect the activation of synaptic GABA-A receptors, in the presence of the NMDA receptor antagonist (APV, 40 μM), the AMPA receptor antagonist (DNQX, 10 μM), and the sodium channel blocker (TTX, 0.5 μM) (Fig. 4). Representative traces and ensemble average mIPSCs for each condition are shown in Figure 4A-B. The average mIPSC from each cell was best fit with a double-exponential decay curve, depicted as τ_1 and τ_2. A mean weighted decay constant τ_w was also derived from τ_1 and τ_2 (see Materials and Methods). Table 1 showed the characteristics of mIPSCs modulated by GABA, GX, or Zn^{2+}. GX significantly potentiated the amplitude, decay time τ_2, and the mean weighted decay time τ_w of mIPSCs from DGGCs (Fig. 4C−D). However, Zn^{2+} perfusion did not change GX-modulated mean peak amplitude or decay time constant. These findings indicate that Zn^{2+} blockade of GX- induced GABAergic currents is highly selective for extrasynaptic δGABA-A receptor-mediated tonic currents.

3.4. The Zn^{2+} chelator TPEN reverses Zn^{2+} blockade of GX-sensitive tonic currents

To further confirm the Zn^{2+} blockade of extrasynaptic δGABA-A receptors, we investigated tonic current response in the presence of a membrane-permeable, high affinity Zn^{2+} chelator, N,N,N',N'-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN). Zn^{2+} (100 μM) perfusion blocked GX-potentiated tonic currents in DGGCs, resulting in a positive shift of tonic current density (0.24 ± 0.09 pA/pF). Such blockade by Zn^{2+} was prevented when TPEN (100 μM) was added to the perfusion, and DGGCs displayed −0.27 ± 0.11 pA/pF negative shift in tonic current density (Fig.5). The difference between Zn^{2+}-antagonized and TPEN-enhanced tonic currents achieves statistical significance ($p = 0.008$, $n = 5$ cells per group). Without exogenous Zn^{2+} added to perfusion, TPEN sustained the negative shift of current density modulated by GX (−0.2 ± 0.08 pA/pF, $n = 5$ cells), indicating significant modulation of endogenous Zn^{2+} within the hippocampus.

3.5. Intrahippocampal Zn^{2+} antagonizes the seizure protective effect of GX in fully-kindled mice

To directly investigate the interactions of Zn^{2+} and GX on hippocampal excitability and seizures, we examined the antiseizure activity of GX with or without intrahippocampal Zn^{2+} infusion in a mouse kindling model, in which the fully-kindled mice exhibit consistent, generalized stage 5 seizures. The experimental paradigm was shown in Fig. 6A.

Intrahippocampal Zn^{2+} (10–300 μM) infusion was performed 10 min before GX (10 mg/kg, s.c.) treatment followed by kindling stimulation. GX (10 mg/kg, s.c.) significantly suppressed kindling-induced seizures (Fig. 6D-E). Zn^{2+} infusion blocked the antiseizure activity of GX dose-dependently, in which Zn^{2+} at 300 μM achieves a statistical significance. The electrograph recordings of electrically-induced, kindling afterdischarge (AD) were shown in Fig. 6B. GX-treated mice (10 mg/kg, s.c.) displayed significantly reduced AD durations compared to vehicle-treated mice (Fig. 6C). Despite GX treatment, 300 μM Zn^{2+}-infused mice exhibited significantly greater AD durations and higher incidence of seizures (Fig. 6C−E). The reversible effect of Zn^{2+} on kindling seizures was demonstrated since all animals exhibited stage 5 seizures after 24-hr wash-out of drug. Overall, these results suggest that Zn^{2+} plays a key role in the modulation of seizure susceptibility by anticonvulsant neurosteroids like GX that are potent activators of δGABA-A receptors.

4. Discussion

Zn^{2+} is an extremely abundant transition metal in the synaptic vesicles of hippocampal glutamatergic mossy fibers and remains a key factor in the modulation of neuronal plasticity (Assaf and Chung, 1984). Disruption of Zn^{2+} homeostasis is associated with many neurological disorders, including seizures, epilepsy, and conditions with compromised brain functions. Previous studies show that Zn^{2+} modulates kinetics of synaptic GABA-A receptors and alters the balance of inhibition and excitability (Barberis et al., 2000; Lambert and Belelli, 2002). Zn^{2+} exhibits higher sensitivity to extrasynaptic δGABA-A receptors than synaptic γGABA-A receptors (Hosie et al., 2003; Wei et al., 2003). However, its inhibition on extrasynaptic receptor-mediated tonic currents and interactions with neurosteroids are not fully understood. Our current study shows that Zn^{2+} pretreatment prevents the anticonvulsant activity of neurosteroids, an effect most likely due to the Zn^{2+}blockade of neurosteroid-potentiated extrasynaptic δGABA-A receptors. These receptors represent the main contributors in maintaining the tonic inhibition in the dentate gyrus, which is involved in a number of seizure and memory disorders (Carver et al., 2016).

Positive allosteric modulators targeting extrasynaptic δGABA-A receptors, such as neurosteroids, are being evaluated as potential therapeutic agents for the treatment of hyperexcitable brain disorders (Reddy and Rogawski, 2001; 2010; Reddy et al., 2018; Younus and Reddy, 2018). GX is a neurosteroid analog developed as a more favorable therapeutic compound with superior bioavailability and pharmacokinetic profile compared to its prototype neurosteroid AP. Synthetic GX serves as a robust antiseizure agent as well as a powerful allosteric modulator of GABA-A receptors with higher selectivity for extrasynaptic δGABA-A receptors (Reddy and Rogawski, 2000; 2010; Clossen and Reddy, 2017a; b; Chuang and Reddy, 2018). Elucidating the neuroprotective effects of GX as well as its interactions with other molecules at the δGABA extrasynaptic receptors is of critical importance for clinical use as an antiepileptic drug. In the present study, we demonstrate that Zn^{2+} selectively blocks GX-induced tonic inhibition, but not phasic inhibition in DGGCs. Chelation of endogenous Zn^{2+} sustains the enhancement of tonic inhibition by GX. Furthermore, intrahippocampal infusion of Zn^{2+} significantly blocked the antiseizure activity of GX in the hippocampus kindling model of epilepsy. Zn^{2+} selective antagonistic

interactions with GX at the extrasynaptic δGABA-A receptors in the hippocampus may contribute to the blockade of GX-induced antiseizure activity by Zn^{2+}; this drug-drug interaction provides clinical implications in the therapeutic use of GX. Overall, these findings are compatible with an excitability-facilitating and proconvulsant role of Zn^{2+} in seizure- related disorders (Buhl et al., 1996; Cavazos et al., 1991; Coulter, 2000).

Receptor subunit composition plays a critical role not only in neurosteroid sensitivity but also in Zn^{2+} inhibition on GABA-A receptors (Draguhn et al., 1990; Smart et al., 1991). Although, in recombinant systems, Zn^{2+} inhibition on GABA-A receptors exhibits similar sensitivity in human α4β3γ2 and α4β3δ GABA-A receptors (Brown et al., 2002), Zn^{2+} significantly shortens the decay time constant of spontaneous IPSCs as well as dendritically-evoked IPSCs in wildtype δ-rich DGGCs but not δ-subunit knockout DGGCs from hippocampus slices (Wei et al., 2003). The absence of sensitivity to Zn^{2+} inhibition in δ-subunit knockout DGGCs may be due to the compensatory upregulation of γ2 subunits and thus the reduction of Zn^{2+} binding sites (Peng et al., 2002; Hosie et al., 2003; Carver and Reddy, 2013). In the present study, we compared the sensitivity of GABA-induced currents and GX-potentiated GABA chloride currents to Zn^{2+} inhibition in native δ-abundant DGGCs and δ-sparse CA1PCs. We found increased inhibition of neurosteroid-potentiated GABA currents by Zn^{2+} in DGGCs compared to CA1PCs, displaying the selectivity of Zn^{2+} inhibition based on receptor subunit composition. In addition, Zn^{2+} at 30 μM completely blocks GX-potentiated GABAergic chloride currents, similar to that of the competitive antagonist, bicuculline and gabazine. Due to its preferential modulation to δ- containing receptors, Zn^{2+} represents a potential noncompetitive antagonist of extrasynaptic δGABA-A receptors and would aide in pharmacological investigations of extrasynaptic GABA-A receptors.

In single channel studies, Zn^{2+} primarily inhibits GABA-A receptors through the reduction of channel opening probability (Smart, 1992; Smart et al., 1994). In slice recordings, Zn^{2+} significantly reduces phasic mIPSC event amplitude and kinetics, as well as desensitization kinetics (Barberis et al., 2000; Ruiz et al., 2004). However, we demonstrate that GX-enhanced phasic mIPSCs were unaltered by Zn^{2+}. It could be that GX potentiation overcomes Zn^{2+} depression of synaptic receptors so that the overall effects of Zn^{2+} on GX-potentiated phasic currents were undetectable. However, in tonic current potentiation, Zn^{2+} blocks GX-potentiated tonic response concentration-dependently in δ-rich DGGCs, probably due to its greater sensitivity towards δ-containing receptors. Unexpectedly, Zn^{2+} prolongs the fast decay time constant τ1 of mIPSCs in the presence of GX. One possibility might be due to the biphasic effects of Zn^{2+} on GABA-B receptors that tonically enhance GABA-A receptor currents (Turgeon and Albin, 1992; Khatri et. al., 2018). In addition, receptor combinations with different subunits respond to Zn^{2+} and neurosteroids differently. Finally, Zn^{2+} may have different effects on fast- closing GABA-A receptor subtypes (Draguhn et al., 1990; Smart et al., 1991).

Zn^{2+} has been shown to act on multiple neurotransmission systems in the brain. In addition to its inhibitory effects on GABA-A receptor function (Smart et al., 1991;1994; Barberis et. al., 2000; Carver et al., 2016), Zn^{2+} also antagonizes excitatory glutamate NMDA receptor channels and decreases the activation and surface expression of NR2A-contaning NMDA

receptors in hippocampal neurons (Westbrook and Mayer, 1987; Vogt et al., 2000; Zhu et al., 2012). Additionally, Zn^{2+} enhances the activity of AMPA receptors (Timofeeva and Nadler, 2006) and increases glycinergic neurotransmission. Therefore, it is possible that the excitability-facilitating and proconvulsant role of Zn^{2+} against GX-induced protective effects in the kindling seizure model may be resulted from the overall outcome of disrupted neurotransmission by Zn^{2+}. Nevertheless, we have previously shown a critical role of extrasynaptic δGABA-A receptor in seizure susceptibility as mice lacking δ subunit have significantly reduced tonic currents and greater seizure susceptibility (Carver et al., 2014). Thus, Zn^{2+} selective antagonistic interactions with GX at the extrasynaptic δGABA-A receptors in the hippocampus may contribute to the inhibition of GX-induced antiseizure activity by Zn^{2+}.

Zn^{2+} exhibits antagonistic activity at α4βγ-and α1βδ-containing GABA-A receptors, albeit at lower sensitivities than α4βδ subtypes (Brown et al., 2002). Specific deletion of δ subunits in α1βδ- containing DG interneurons leads to the reduced GABAergic tonic inhibition and elevated firing rate of interneurons that decrease DGGC excitability and in vivo seizure susceptibility (Lee and Maguire, 2013). Therefore, Zn^{2+} may also contribute to the disinhibition of α1βδ-containing interneurons. In the seizure model of hippocampal kindling, the composition and expression of GABA-A receptors in the hippocampus has been largely altered (Nishimura et al., 2005). In hippocampus DG, the mRNA levels of δ subunit were significantly reduced with an increase in α2 subunits seven days after kindling completion. In CA3 pyramidal neurons, α2 and β3 subunits were significantly upregulated after hippocampal kindling. While in CA1 pyramidal neurons, no significant changes of GABA-A receptor subunits were observed. As a result, the sensitivity of each cell types to Zn^{2+} modulation may be different from animals without kindling, which may also involve in the Zn^{2+} antagonism of GX-induced seizure protection. Nevertheless, we observed the net outcome of Zn^{2+} in hippocampal kindling to be proconvulsant.

Zn^{2+} increases the permeability of blood-brain barrier and proconvulsant activity in rats (Yorulmaz et al., 2013). Severe blood-brain barrier damage is also found in PTZ-induced epileptic seizures. The Zn^{2+} level in the brain may fluctuate due to the contribution of peripheral Zn^{2+} that penetrates the damaged blood-brain barrier. Zn^{2+} concentrations are particularly high in some brain regions including hippocampus and amygdala, which are also the most vulnerable regions for the focal point of seizures (Frederickson et al., 2005). The effective concentrations of Zn^{2+} in the inhibition of GX-potentiated tonic currents are within the levels that may occur in synaptic clefts following the Zn^{2+} release from the vesicles of presynaptic neurons during neuronal activity. Therefore, Zn^{2+} may disrupt the neurosteroid-augmented basal inhibitory tone and hinder the balance of hippocampal neural circuits. In physiological condition, free zinc cations released from glutamatergic synapses in the hippocampus may block the inhibition of extrasynaptic activity of neurosteroids, hindering the antiseizure effects of neurosteroids. Therefore, combination therapies of neurosteroids with Zn^{2+} chelators may be potential avenues for the treatment of seizure-related disorders. We have attempted to examine the effects of Zn^{2+} chelators on the seizure activity of hippocampal kindling animals. However, a previous study shows that TPEN exerts notable toxic effects in *in vivo* animal studies, which was the reason these studies were not conducted further (Elsas et al., 2009). In Foresti et al., 2008, they used another

Zn^{2+} chelator and found that pretreatment of Zn^{2+} chelator DEDTC (700 mg/kg) diminishes the duration of behavioral seizures and electrical afterdischarges, and EEG spikes, without altering seizure severity progression in a rat model of amygdala kindling, showing the antiseizure effects of the Zn^{2+} chelator (Foresti et al., 2008). Although their experiments show an antiseizure role of the zinc chelator, they cannot exclude the potential toxic effects of DEDTC. In their studies, they selected a dose that did not cause significant behavioral changes but still may be high enough to cause toxic effects in cellular and molecular levels since animals experienced severe ocular and nasal bleeding and reduced locomotor activity with the treatment of DEDTC. Future studies of zinc chelators on the seizure activity are feasible when nontoxic Zn^{2+} chelators become available.

The schematic diagram of the antagonistic interactions of Zn^{2+} and neurosteroids (NSs) at GABA-A receptors is summarized in Fig. 7. Neurosteroids exhibit powerful seizure protective effects against experimental seizures probably through the potentiation of synaptic and extrasynaptic GABA-A receptor-mediated inhibitory currents. However, Zn^{2+} selectively hinders neurosteroid-augmented tonic inhibition but not phasic inhibition, which may partially contribute to the Zn^{2+} antagonism of neurosteroid-induced antiseizure activity. Previous studies show that Zn^{2+} blocks the frequency of mIPSCs in DGGCs from kindled but not controls (Buhl et al., 1996). Reduced Zn^{2+} sensitivity in GABA currents in DGGCs from rats with status epilepticus (Kapur and Macdonald, 1997). Zn^{2+} inhibition of GABA-A receptor function is also decreased in pilocarpine-induced status epilepticus (Banerjee et al., 1999). Overall, Zn^{2+} greatly contributes to the balance of neuronal excitation and inhibition in both physiological and pathophysiological conditions. There are many questions that remain unclear, including whether posttranslational modifications affect Zn^{2+} sensitivity of GABA-A receptors.

In conclusion, the present study demonstrates selective antagonistic interactions of Zn^{2+} and GX at extrasynaptic δGABA-A receptors in the hippocampus. Zn^{2+} chelation sustains the potentiation of tonic inhibition by GX. Furthermore, intrahippocampal infusion of Zn^{2+} significantly blocked the antiseizure activity of GX in the mouse kindling model of epilepsy, indicating that Zn^{2+} reduces the antiseizure effects of neurosteroids by selective blockade of extrasynaptic δGABA-A receptors. These pharmacodynamic interactions may have clinical implications in neurosteroid therapy of brain disorders susceptible to zinc fluctuations.

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Highlights:

- Zinc modulates hippocampus neuronal excitability.
- Neurosteroids are powerful anticonvulsants via modulation of GABA-A receptors
- Zinc selectively blocked extrasynaptic tonic inhibition.
- Zinc prevented the protective effects of ganaxolone, an antiepileptic synthetic neurosteroid.
- Zinc−neurosteroid interactions have therapeutic implications in epilepsy.

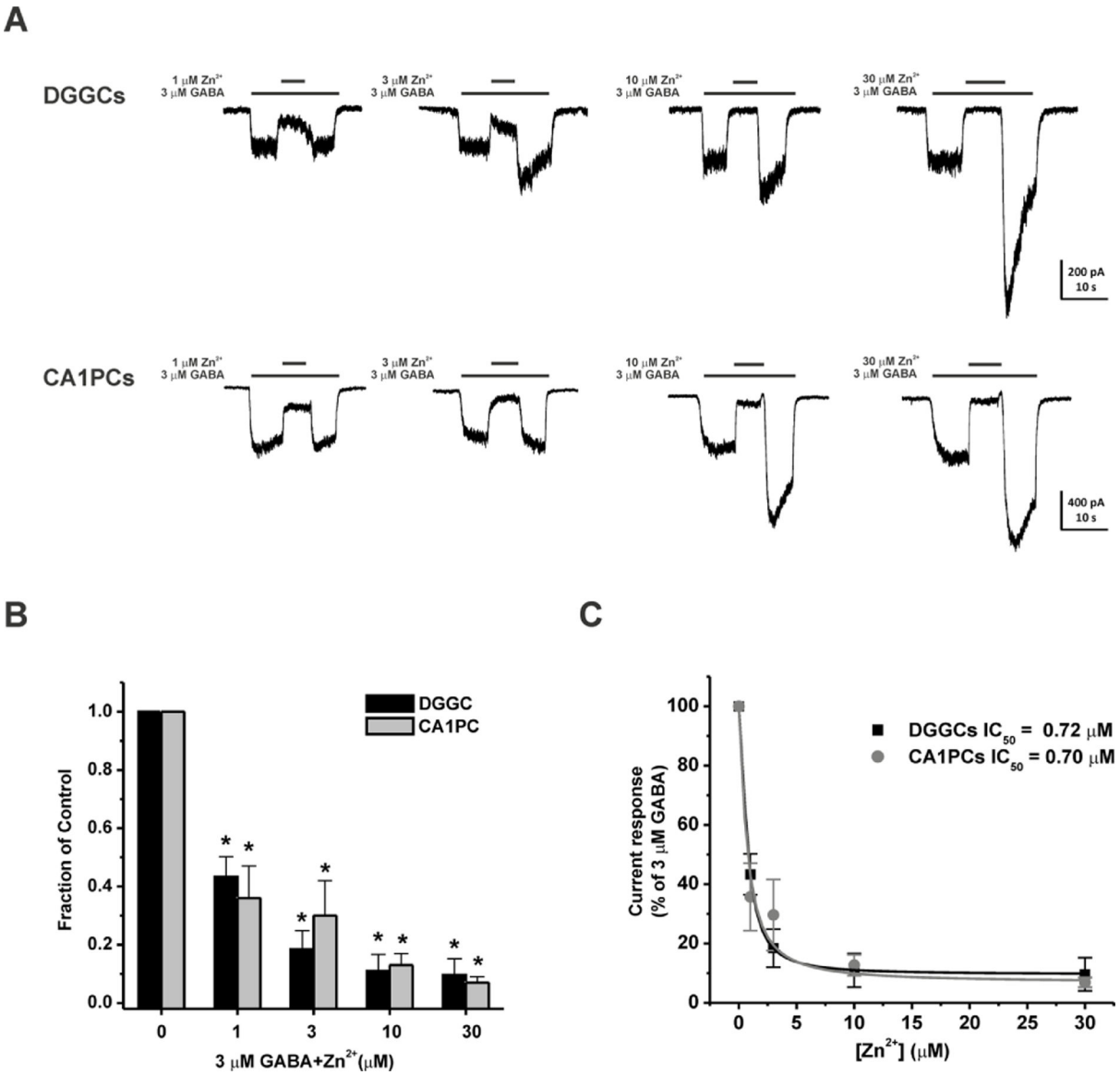

Fig. 1.

Zn^{2+} blockade of GABA-gated currents is concentration-dependent in dissociated DGGCs and CA1PCs. (A) Representative whole-cell GABAergic current recording from DGGCs and CA1PCs. Zn^{2+} (1–30 μM) inhibited GABA currents in a concentration-dependent fashion. (B) Fractional block of currents by Zn^{2+} (1–30 μM). (C) Inhibitory concentration response curves of DGGCs and CA1PCs by Zn^{2+}. DGGCs IC_{50} = 0.72 μM; CA1PCs IC_{50} = 0.70 μM. *p<0.05 vs. control. Each bar represents mean ± SEM (n = 4–6 cells per group).

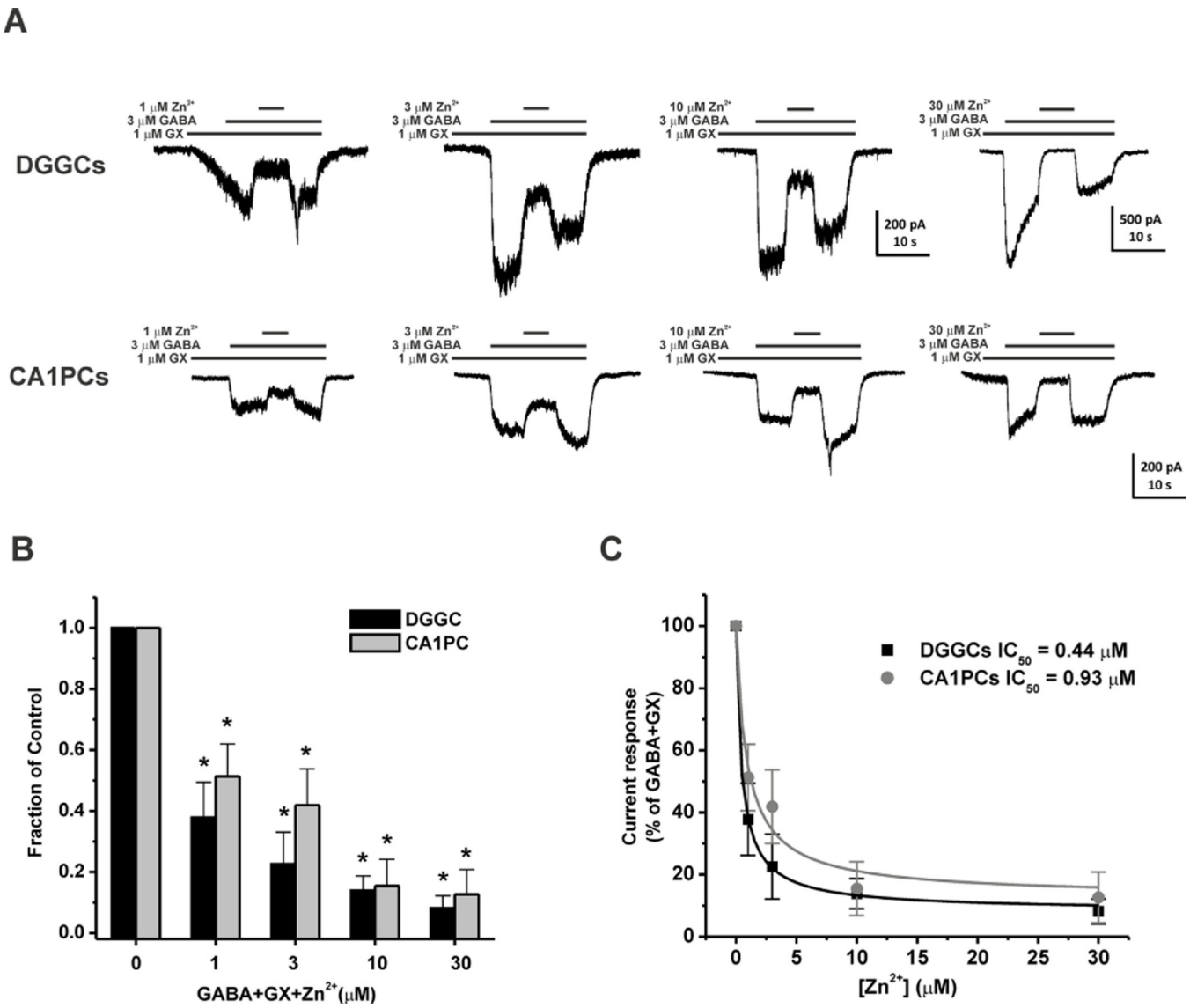

Fig. 2.

Zn^{2+} blockade of GX-sensitive GABA-gated currents is concentration-dependent in dissociated DGGCs and CA1PCs. (A) Representative whole-cell GX-activated GABAergic current recording from DGGCs and CA1PCs. Zn^{2+} (1–30 μM) inhibited GX-potentiated GABA currents in a concentration-dependent fashion. (B) Fractional block of currents by Zn^{2+} (1–30 μM). (C) Inhibitory concentration response curves of DGGCs and CA1PCs by Zn^{2+}. DGGCs IC_{50} = 0.44 μM; CA1PCs IC_{50} = 0.93 μM. *p<0.05 vs. control. Each bar represents mean ± SEM (n = 4–5 cells per group).

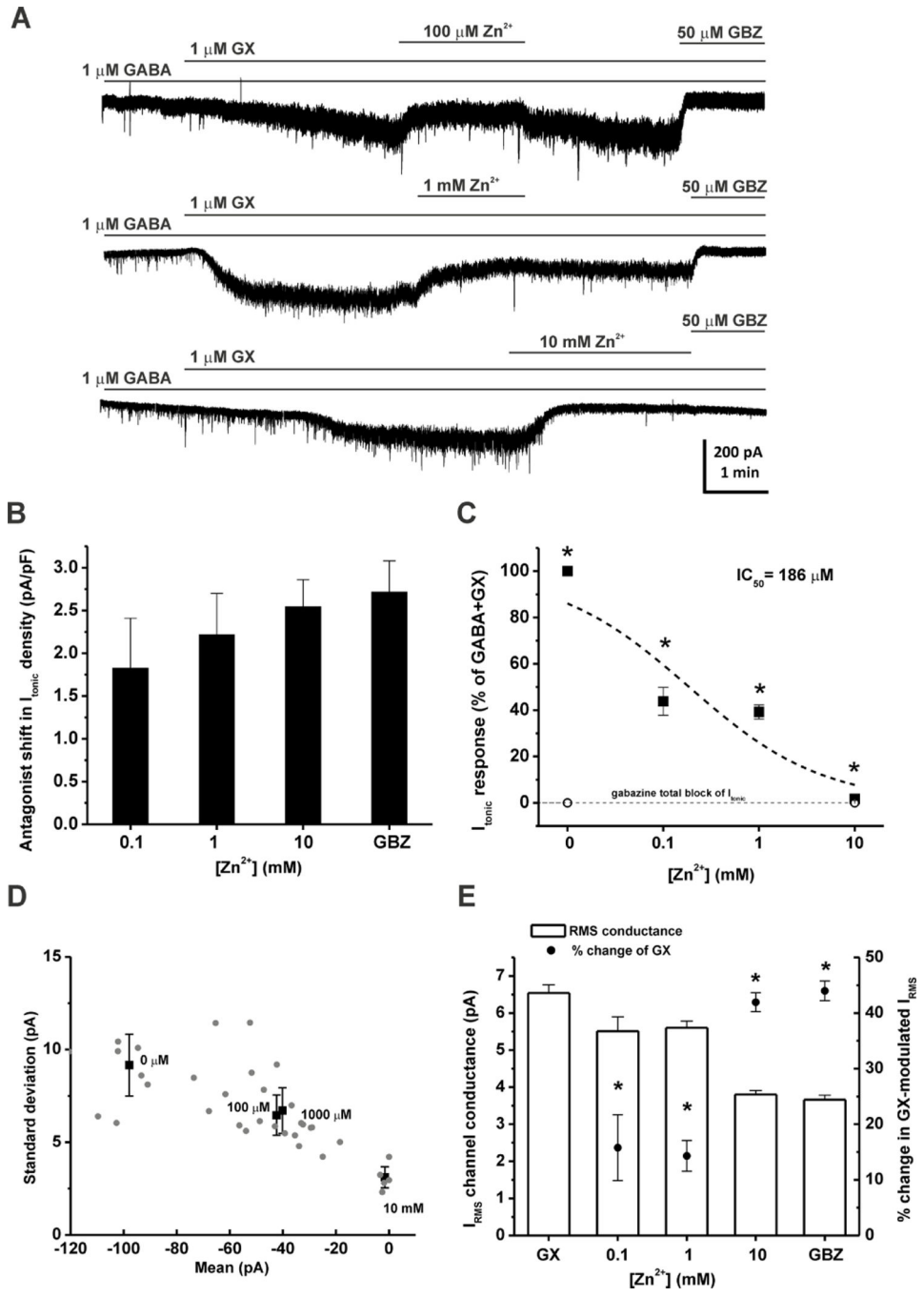

Fig. 3.

GX-potentiated tonic currents are sensitive to Zn^{2+} blockade in DGGCs in hippocampus slices. **(**A) Representative GABAergic I_{tonic} recording from DGGCs in the presence of GABA + GX, Zn^{2+}, and 50 μM gabazine (GBZ). (B) Concentration-response of GX-modulated, normalized I_{tonic} (pA/pF) to block by Zn^{2+} (0.1–10 mM). (C) Fractional response of GX-modulated I_{tonic} due to Zn^{2+} (*$p<0.05$ vs. maximal block due to saturating 50 μM gabazine (GBZ). (D) Zn^{2+} reduced the mean and standard deviation of GX-potentiated tonic currents in a concentration- dependent fashion. (E) I_{RMS} channel

conductance (pA) and % change of Zn^{2+} blockade of GX- dependent I_{RMS}. GX denotes 1 μM GABA + 1 μM GX condition without Zn^{2+}. *p<0.05 vs. GX. Each bar represents mean ± SEM (n = 6–21 cells per group).

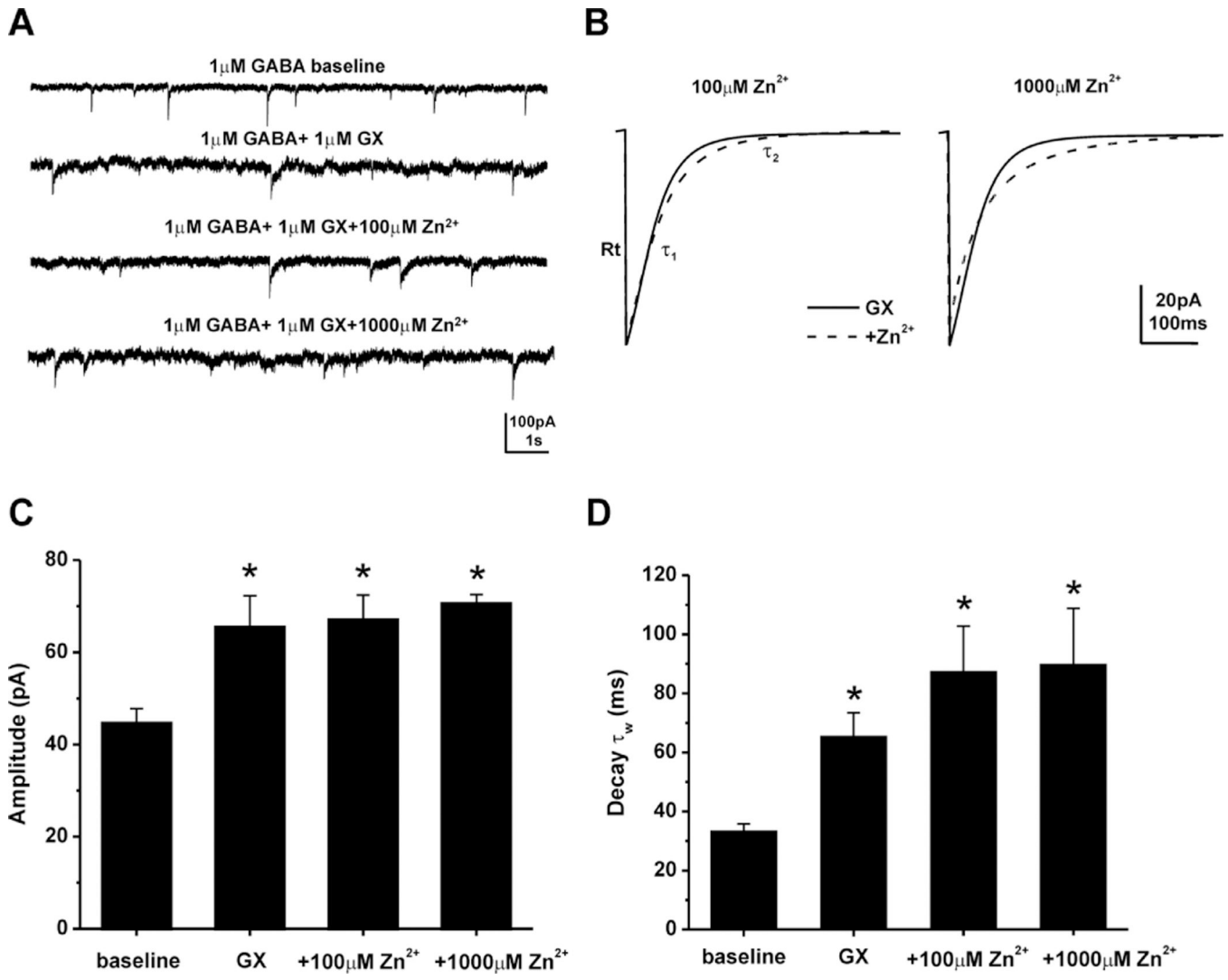

Fig. 4.

GX-activated mIPSCs are not sensitive to Zn^{2+} blockade. (A) Representative traces of mIPSCs. $GABA_A$ receptor mIPSC activity was isolated by using TTX, APV, and DNQX, and was completely blocked by gabazine. GX (1 μM) potentiated synaptic current but Zn^{2+} did not significantly alter the GX-potentiated mIPSCs. (B) Averaged mIPSCs in the presence of GX (solid line) and addition of Zn^{2+} (dashed line). (C) Amplitudes were not significant different between GX and 100 μM Zn^{2+} ($p = 0.65$), or GX and 1000 μM Zn^{2+} ($p = 0.70$). (D) Zn^{2+}did not significantly alter mean weighted decay kinetics (τ_w) of GX modulation. Data bars represent mean ± SEM (n = 4–9 cells per group). *$p<0.05$ vs. baseline. *GX denotes GABA + GX; baseline denotes GABA alone; Rt denotes rise time.*

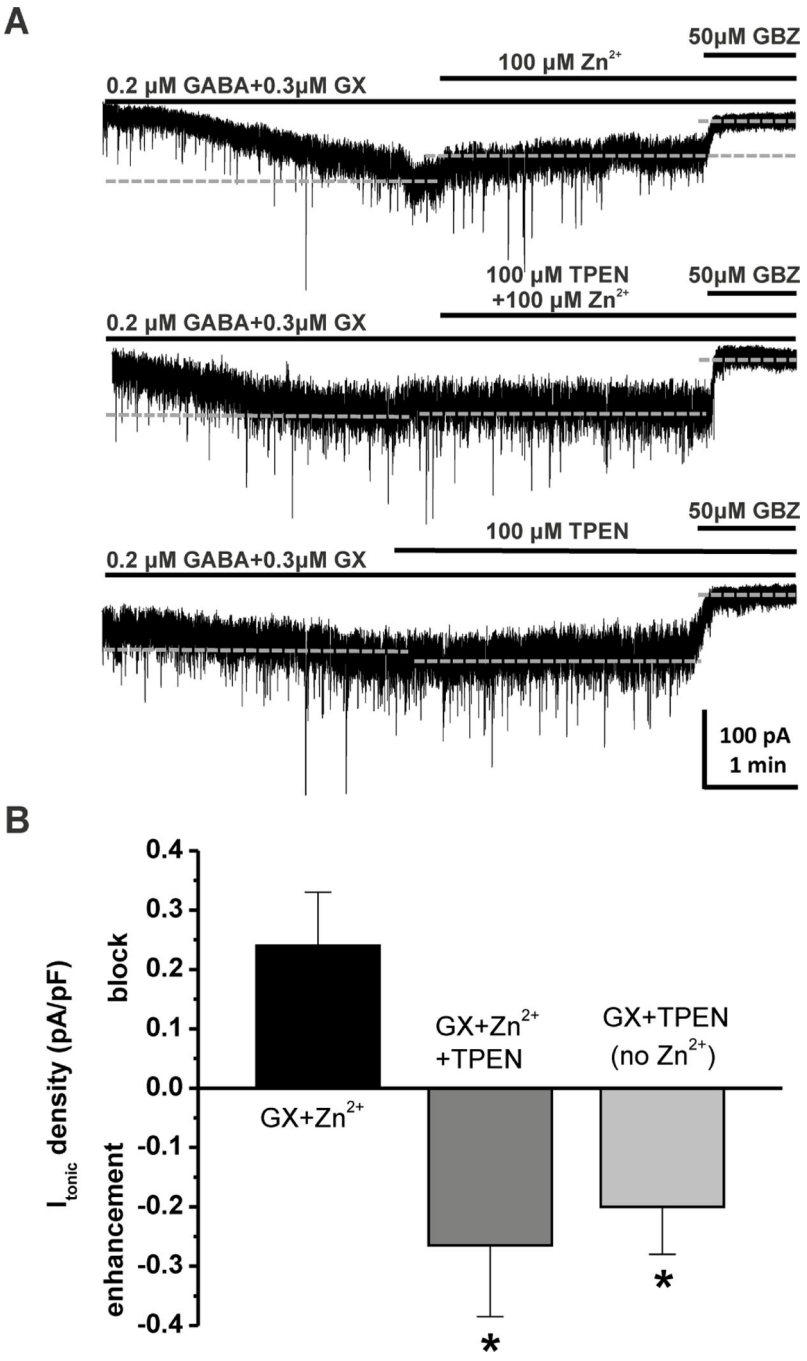

Fig. 5.

Zn^{2+} chelator TPEN inhibits the Zn^{2+} antagonism of GX-sensitive tonic currents. (A) Representative GABAergic I_{tonic} recording from DGGCs before and after the application of Zn^{2+} (100 μM) or Zn^{2+} chelator TPEN (100 μM), or in coapplication of both in modulation of 0.2 μM GABA + 0.3 μM GX. TPEN prevented Zn^{2+} blockade of GX-sensitive current, and I_{tonic} exhibited significant enhancement by TPEN in hyperpolarization of the holding current level. Quantification of I_{tonic} was achieved relative to complete block by gabazine (GBZ). Dashed lines indicate average holding current level throughout each drug

application. (B) I_{tonic} density shift (pA/pF) in the presence of Zn^{2+} and/or TPEN, with positive values as block of GX-sensitive I_{tonic} and negative values as enhancement of I_{tonic}. Data are mean ± SEM (n = 5 cells per group). *$p<0.05$ vs. GX + Zn^{2+} combination (Mann-Whitney U-test).

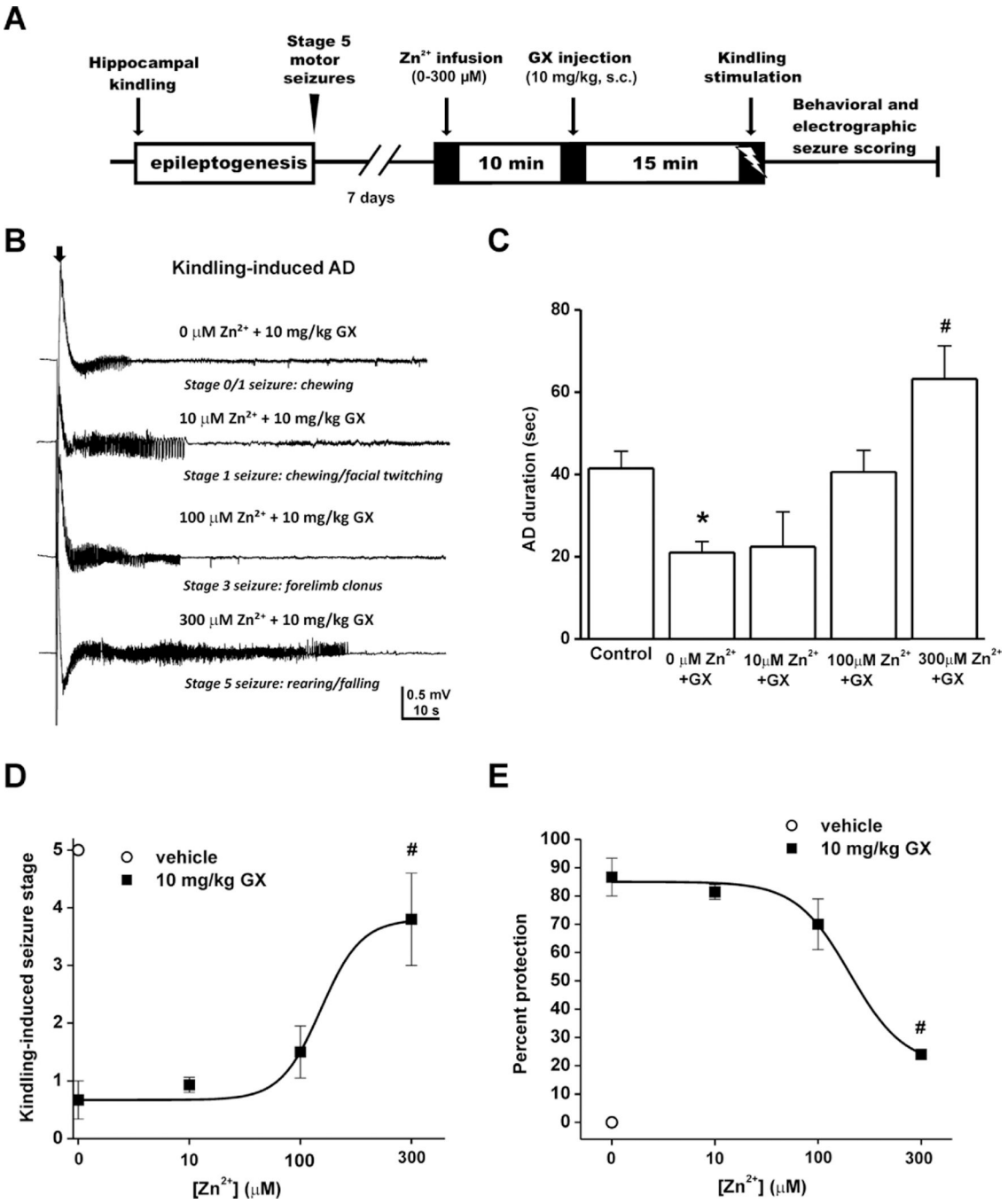

Fig. 6.

Intrahippocampal Zn^{2+} infusion completely prevents the antiseizure effect of the neurosteroid GX in fully-kindled mice. (A) Experimental paradigm and infusion protocol for saline or Zn^{2+} delivery (0–300 μM) prior to GX (10 mg/kg, s.c.) treatment and kindling stimulation. (B) Electrograph recordings of electrically-induced, kindling afterdischarge (AD). The black arrow denotes stimulation-onset artifact. (C) AD duration of electrograph activity. Control denotes saline infusion (Zn^{2+} -free) and vehicle injection (GX-free) condition upon kindling. (D) Kindling- induced behavioral seizure score after Zn^{2+} infusion.

ED_{50} = 150 μM. (E) Dose-response curve of percent seizure protection. Anticonvulsant effect of 10 mg/kg GX was inhibited by 300 μM Zn^{2+}. After 24 hr wash-out, mice displayed stage 5 seizures and similar AD duration to the control group. Data represent mean ± SEM (n = 5–6 mice per group). *p<0.05 vs. control; #p<0.05 vs. GX without Zn^{2+} (Mann-Whitney U-test).

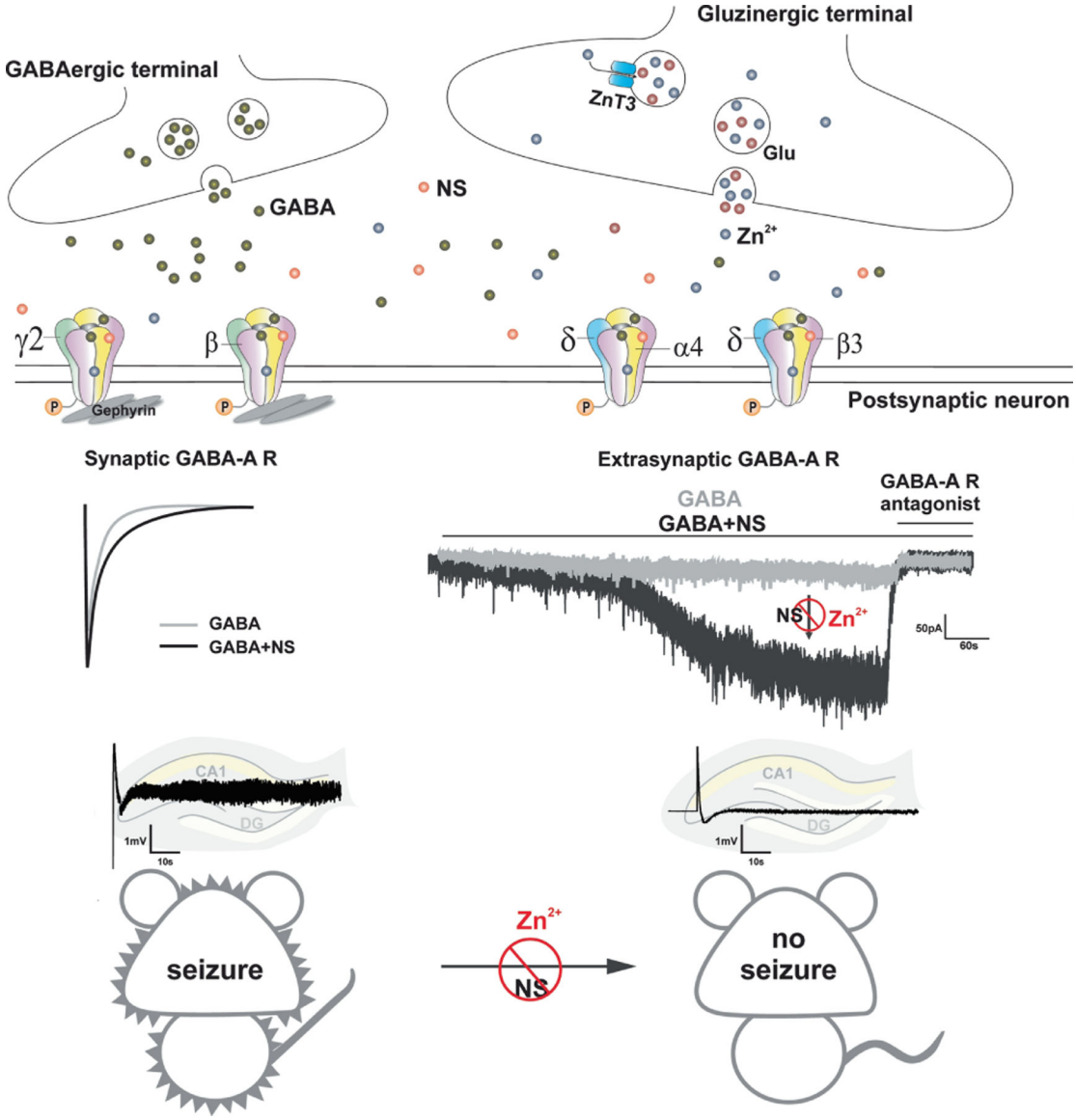

Fig. 7.

Schematic diagram of pharmacodynamic interactions of Zn^{2+} and neurosteroids at GABA-A receptors. Neurosteroids exhibit powerful seizure protective effects against kindling-induced limbic seizures probably through the potentiation of synaptic and more preferably extrasynaptic GABA-A receptor-mediated inhibitory currents. Zn^{2+} selectively hinders neurosteroid-sensitive tonic inhibition but not phasic inhibition, which may partially contribute to the Zn^{2+} antagonism of antiseizure activity of neurosteroids, including endogenous neurosteroids

Table 1

GABA-A receptor-mediated mIPSC characteristics in DGGCs.

Group (n)	Amplitude (pA)	Rise$_{10–90\%}$ (ms)[a]	Decay τ_1 (ms)[a]	Decay τ_2 (ms)[a]	Decay τ_w (ms)	Frequency (Hz)
1μM GABA (13)	43.1 ± 1.5	1.1 ± 0.1	10.6 ± 1.3	48.0 ± 1.9	30.2 ± 1.4	0.56 ± 0.08
GABA+1μM GX (9)	65.4 ± 6.9 *	1.6 ± 0.3	14.5 ± 1.5	104.8 ± 10.7 *	65.5 ± 7.9 *	0.43 ± 0.16
GABA+GX+100μM Zn^{2+} (5)	67.0 ± 5.4 *	1.0 ± 0.3	22.4 ± 4.7 *#	114.1 ± 16.1 *	87.4 ± 15.4 *	0.30 ± 0.17
GABA+GX+1000μM Zn^{2+} (4)	70.5 ± 2.0 *	1.0 ± 0.1	18.7 ± 3.9 *#	144.9 ± 18.9 *	89.9 ± 18.9 *	0.10 ± 0.02 *

* $p<0.05$ vs. GABA

# $p<0.05$ vs. GABA+GX.