Modulation of inhibitory and excitatory neurotransmissions by Zn$^{2+}$ on the substantia gelatinosa neurons of the trigeminal subnucleus caudalis in mice

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Abstract. The substantia gelatinosa of the trigeminal subnucleus caudalis has been considered to be an essential location for the transference of orofacial sensory signals. The co-localization of inhibitory and excitatory neurotransmitters in the same substantia gelatinosa (SG) neurons has demonstrated their essential part in the modification of nociceptive transmission. Zn$^{2+}$ is particularly numerous in the mammalian central nervous system. There are proofs demonstrating the role of Zn$^{2+}$ in the modulation of voltage- and ligand-gated ion channels. However, little is known about what roles Zn$^{2+}$ may play in the modulation of signal transmission in the SG neurons of the trigeminal subnucleus caudalis (Vc). Therefore, in this study, we used the whole-cell patch clamp technique to find out the effect of Zn$^{2+}$ on the responses of three main neurotransmitters (glycine, GABA, and glutamate) on SG neurons of the Vc in mice. We have proved that Zn$^{2+}$ induces a big potentiation of glycine receptor-mediated response but attenuates GABA- and glutamate-induced responses at micromolar concentrations, however, enhances glutamate-induced response at nanomolar concentration. Taken together, these data demonstrated that Zn$^{2+}$ can modulate glycine, GABA and glutamate-mediated actions on the SG neurons of the Vc and support an important mechanism in spinal sensory information signaling.

Key words: Substantia gelatinosa — Patch clamp techniques — Zn$^{2+}$

Introduction

The substantia gelatinosa (SG, lamina II) of the trigeminal subnucleus caudalis (Vc, also called the medullary dorsal horn) has been considered to be essential location for the transference of orofacial sensory signals, because it receives the nociceptive events from primary afferents, including thin myelinated Aδ- and unmyelinated C-fibers (Light and Perl 1979; Todd 2002; Santos et al. 2007). Glycine and γ-aminobutyric acid (GABA) are major inhibitory neurotransmitters, whereas glutamate is mainly an excitatory neurotransmitter. The co-localization of inhibitory and excitatory neurotransmitters in the same SG neurons has demonstrated their essential part in the modification of nociceptive transmission (Todd et al. 1996; Kohno et al. 1999; Price et al. 2005). For this reason, if any compound alters the functional properties of neurotransmitters in the SG neurons, it may modify significantly the pain-signaling messages proceeding from orofacial region to the brain.
Zn$^{2+}$, known to be a necessary nutrient, is the second most plentiful trace element in the human body and has a fundamental effect on cellular growth, division, and differentiation (Vallee and Falchuk 1981; Coleman 1992). Among all transition metals, Zn$^{2+}$ is also particularly numerous in the mammalian central nervous system (CNS) and is localized with a high concentration in the neuronal parenchyma (Frederickson et al. 1987; Frederickson 1989). This divalent element is also a required factor necessary for the normal operation of the nervous system (Hurley and Shrader 1972). However, paradoxically, at higher concentrations, it may serve as a neurotoxin that leads to some pathological brain diseases (Choi et al. 1988; Duncan et al. 1992; Gower-Winter and Levenson 2012).

There is much evidence demonstrating the role of Zn$^{2+}$ in the modulation of voltage- and ligand-gated ion channels. For example, in the third-order neurons isolated from the crucian carp retina, Zn$^{2+}$ was detected to modulate both glycine receptors and GABA receptors (Li and Yang 1999). In addition, Zn$^{2+}$ also acts as an inhibitory neuromodulator for the release of glutamate receptors in the rat hippocampus (Takeda et al. 2003). However, little is known about the roles that Zn$^{2+}$ may play in the modulation of signal transmission in the SG neurons of the Vc. Therefore, in this study, we used the whole-cell patch clamp technique to find out the effect of Zn$^{2+}$ on the responses of three main neurotransmitters (glycine, GABA, and glutamate) on SG neurons. 

Materials and Methods

Animal and brain slice preparation

All experiments on living animals were ratified by the Experimental Animal Care and Ethics Committee of Chonbuk National University. Immature male and female Institute of Cancer Research (ICR) mice (7–20 postnatal days) (Damul Science, Suwon, Korea) tested in this study were housed under a stable environment including the 12-hour light/dark cycles (lights on at 06:00) with access to water and food ad libitum.

We used the same method to prepare brain slices as in our previous study (Nguyen et al. 2015). Firstly, ICR mice were beheaded; the brains were removed quickly and placed in ice-cold bicarbonate-buffered artificial cerebrospinal fluid (ACSF) containing (in mM): 126 NaCl, 2.5 KCl, 2.4 CaCl$_2$, 1.2 MgCl$_2$, 11 D-glucose, 1.4 NaH$_2$PO$_4$, 25 NaHCO$_3$ and 0.5 sodium ascorbate (pH 7.3–7.4, bubbled with 95% O$_2$ and 5% CO$_2$). The brains were cut into coronal slices (180–200 μm in thickness) containing the Vc by a vibratome (VT1200S, Leica Biosystem, Nussluch, Eisfeld, Germany) in ice-cold ACSF and kept in oxygenated ACSF at room temperature for at least one hour before electrophysiological recording.

Electrophysiology and data analysis

Each individual brain slice was moved into the recording chamber. There, it was continuously submerged and perfused with oxygenated ACSF at a flow speed of 4–5 ml/min. To observe the slices, we used an upright microscope (BX51WI, Olympus, Tokyo, Japan) consisting of some Nomarski differential interference contrast optics. The SG (lamina II) of the medullary dorsal horn was identified as a translucent band that was medial to the spinal trigeminal tract and went along the lateral sides of the slice.

The patch pipettes were pulled in a thin-wall borosilicate glass-capillary tubing (PG52151-4, WPI, Sarasota, FL, USA) of the Flaming/Brown puller (P-97, Sutter Instruments Co., Novato, CA, USA). The pipette solution was passed through a disposable 0.22 μm filter and contained the following (in mM): 140 KCl, 1 CaCl$_2$, 1 MgCl$_2$, 10 HEPES, 4 MgATP, 10 EGTA (pH 7.3 with KOH). After the glass-capillary electrode was loaded with the pipette solution, the resistance of the recording pipettes was measured at around 4–6 MΩ. To patch the cell, firstly, a gigaohm seal was formed with SG neuron, then the cell membrane patch was ruptured by negative pressure, and electrical measurement was done using a whole-cell patch-clamp recording mode with an Axopatch 200B (Molecular Devices, CA, USA). The currents of the cell membranes were sampled online using a Digidata 1322A (Molecular Devices, CA, USA) interface linked to a desktop computer. The electrophysiological signals were filtered (2 kHz, Bessel filter of Axopatch 200B) before being digitized at a rate of 1 kHz. The cell holding potential was maintained at ~60 mV throughout the recordings. The acquisition and analysis of the data were done by using Clampex 10.6 software (Molecular Devices, CA, USA). All our recordings were done at room temperature.

Chemicals

Zinc sulfate heptahydrate, glycine, GABA, glutamate, and the chemicals to make ACSF were purchased from Sigma (USA). Stocks of all drugs were prepared according to their solubility in distilled water. We diluted the stock solutions to the desired final concentrations in ACSF just before use and were applied to the neurons via bath application.

Statistics

Software named Origin 7 (OriginLab Corp., Northampton, MA, USA) was used to plot the traces. All values were described in the form of the mean ± SEM. To compare the average amplitudes of inward currents between two groups, we used a paired $t$-test. A $p$-value < 0.05 was recognized as the statistically significant standard.
Results

To investigate whether there were any changes in the response induced by inhibitory or excitatory neurotransmitters in SG neurons, we compared the responses elicited by glycine (30 µM), GABA (30 µM), and glutamate (30 µM) alone and in the presence of Zn²⁺. The cell-voltage clamp recordings were obtained from 43 SG neurons that belonged to 28 ICR mice of 7–20 postnatal days.

Zn²⁺ and glycine

First, we checked the effect of Zn²⁺ on glycine, an inhibitory neurotransmitter. When glycine was successively applied, the inward currents were induced. After that, Zn²⁺ at a low concentration (3 µM) was pretreated alone around five minutes; the Zn²⁺ did not elicit any detectable membrane currents. However, a glycine-induced inward current (I_{Gly}) was strongly potentiated when applied simultaneously with Zn²⁺ (Fig. 1A). As we observed in the bar graph, the mean amplitudes of I_{Gly} alone and in the presence of Zn²⁺ were 59.6 ± 13.5 pA and 149 ± 35.6 pA, respectively (n = 8, p < 0.01, Fig. 1B). Zn²⁺ potentiated these glycine currents when co-applied extracellularly at a concentration of 3 µM.

Besides, it is reported that the physiological extracellular Zn²⁺ concentration is rather in the nanomolar range (Thompson et al. 2000; Kay 2003; Frederickson et al. 2006). Therefore, the effect between Zn²⁺ and glycine was checked in a dose-response manner at different concentration of Zn²⁺ ranging from 10–3,000 nM (Fig. 1C). There was an increase of the I_{Gly} flowing, the rise of Zn²⁺ concentration with an EC₅₀ of 4,093 nM.

Zn²⁺ and GABA

We continued to analyze the effect of Zn²⁺ on another inhibitory neurotransmitter, GABA. As shown in Fig. 2, successive application of GABA 30 µM created a detectable change in membrane current. When Zn²⁺ (3 µM) was treated together with GABA, the GABA-induced inward current (I_{GABA}) was decreased partially (Fig. 2A). The bar graph shows that the mean inward current induced by GABA (85.1 ± 15.7 pA) was reduced to 59.6 ± 19.7 pA in the presence of Zn²⁺ (n = 6, p < 0.01, Fig. 2B). These results indicate that Zn²⁺ at 3 µM concentration inhibits I_{GABA} on SG neurons. Besides, we also evaluated the effect Zn²⁺ in nanomolar concentrations to GABA 30 µM. However, 300 nM Zn²⁺ did not change GABA-mediated responses. There is no significant effect between the mean inward currents induced by GABA alone and in the presence of Zn²⁺ (87.3 ± 15.9 pA and 90.1 ± 14.7 pA, respectively) (n = 9, p > 0.05, Fig. 2C).

Zn²⁺ and glutamate

In the next stage of the experiment, we examined how Zn²⁺ affected the excitatory neurotransmitter of SG neurons, the
glutamate receptors. First, Zn$^{2+}$ was also applied at 3 µM, as in previous experiments. However, in this level of Zn$^{2+}$ concentration, Zn$^{2+}$ did not show any change on glutamate (30 µM)-induced inward current ($I_{\text{Glu}}$) (data not shown). As the Zn$^{2+}$ concentration was increased to 10 µM, the glutamate-activated current was strongly decreased by the simultaneous application with Zn$^{2+}$ (Fig. 3A). The mean amplitude of $I_{\text{Glu}}$ alone (41.8 ± 8.5 pA) was decreased in the presence of Zn$^{2+}$ 10 µM (20.7 ± 4.5 pA) ($n = 7$, $p < 0.01$, Fig. 3B). Again, these results provide evidence that Zn$^{2+}$ inhibits the glutamate-mediated response.

Interestingly, at nanomolar concentration of Zn$^{2+}$, we found that Zn$^{2+}$ (300 nM) increased the $I_{\text{Glu}}$ (Fig. 3C). The mean inward current evoked by glutamate 30 µM in the absence and presence of Zn$^{2+}$ 300 nM were –41.1 ± 10.9 pA and –53.8 ± 14.1 pA, respectively ($n = 6$, $p < 0.05$, Fig. 3D). To summarize all the data between Zn$^{2+}$ and glutamate, these results provide evidence that Zn$^{2+}$ has biphasic effects to glutamate: at the nanomolar concentration (300 nM), Zn$^{2+}$ increases $I_{\text{Glu}}$ but at the micromolar concentration (10 µM), Zn$^{2+}$ inhibits $I_{\text{Glu}}$.

**Discussion**

Zn$^{2+}$ has been known to play many physiological roles in the CNS, including synaptic messenger transmission (Christine and Choi 1990; Xie and Smart 1993), intracellular second messenger pathways (Forbes et al. 1991; Weinberger and Rostas 1991), and functional modulation of ion channels (Winegar and Lansman 1990; Li and Yang 1999). In this study, we used an exogenous Zn$^{2+}$ application in order to examine the physiological role of synaptic Zn$^{2+}$ on amino-acid neurotransmissions. By the electrophysiological approach, we have demonstrated that Zn$^{2+}$ has different effects on different inhibitory and excitatory neurotransmitters in SG neurons of Vc. At a micromolar concentration (3 µM), Zn$^{2+}$ induces a big potentiation of glycine receptor-mediated response but attenuates GABAergic inputs. With glutamate, Zn$^{2+}$ has opposite effects depending on the concentration, Zn$^{2+}$ with micromolar concentration (10 µM), decreases glutamate-induced inward currents but increases them with nanomolar concentration (300 nM).

Growing evidence suggests that released Zn$^{2+}$ can perform as an extracellular modulator of inhibitory and/or excitatory synaptic events (Choi and Koh 1998). Besides, Zn$^{2+}$ can enter postsynaptic neurons through the Ca$^{2+}$-permeable channels and thus exert intracellular effects on physiological signaling functions of ion channels (Weiss et al. 1993; Freund and Reddig 1994; Yin and Weiss 1995). As a signaling substance, an alteration in extracellular Zn$^{2+}$ may change the operation of several membrane channels and neurotransmitters by modifying the transmitter releaser and/or the sensitivity of the postsynaptic cells to transmitter molecules (Harrison and Gibbons 1994; Smart et al. 1994).

Glycine is major fast inhibitory neurotransmitters in the spinal cord that is accumulated in small synaptic vesicles (Burger et al. 1991; Christensen and Fonnum 1991). Glycine receptors are composed of a combination of five distinct transmembrane protein subunits (Pfeiffer et al. 1982). Each receptor subunit includes a large extracellular N-terminal domain and four transmembrane spanning domains (term
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M1-M4), in which the second segment (M2) composes the channel pore-lining $\alpha$-helix (Karlin and Akabas 1995). Glycine-induced currents have been demonstrated to be potentiated by Zn$^{2+}$ at a concentration between 0.1 and 10 µM in third-order neurons isolated from the crucian carp retina, in Xenopus oocytes and human embryonic kidney cells (Laube et al. 1995; Li and Yang 1999; Miller et al. 2005). At low concentrations, this ion metal modulates glycine-mediated currents by increasing the apparent agonist affinity without altering the maximal inducible current (Bloomenthal et al. 1994; Laube et al. 1995). With the results from molecular experiments, it has been concluded that this Zn$^{2+}$ potentiation of glycine-gated currents was specifically mediated by the allosteric signal-transduction processing between ligand binding and channel activation, which involved the key control elements, the residues in the M1-M2 loop and the M2-M3 loop (Lynch et al. 1997, 1998; Miller et al. 2005). Conversely, a higher concentration level of Zn$^{2+}$ (50 µM) significantly inhibited the glycine responses in the cultured rat spinal-cord neurons (Bloomenthal et al. 1994; Laube 2002). Zn$^{2+}$ is a powerful modulator that can increase or decrease the open probability of a glycine channel in a way consistent with a strengthened or impaired affinity of the glycine receptor (Laube et al. 2000).

Another major inhibitory neurotransmitter in the CNS is GABA. These receptors contain some allosteric binding locations for several classes of chemicals that can modulate receptor function (Sivilotti and Nistri 1991; Bowery and Smart 2006). Many studies have demonstrated that Zn$^{2+}$ inhibits GABA$_A$ responses on hippocampal neurons of rats, such as kindled adult hippocampal granule cells (Buhl et al. 1996) and cultured hippocampal neurons (Barberis et al. 2000), as well as on guinea-pig hippocampal neurons (Ruiz et al. 2004). The reduction of GABA$_A$ response by Zn$^{2+}$ obviously depends on the subunit components. GABA$_A$ receptor possessing $\alpha\beta$ subunits

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**Figure 3.** Effect of Zn$^{2+}$ on glutamate-induced inward current ($I_{Glu}$)

**A.** The representative trace showing current evoked by glutamate 30 µM was reduced by Zn$^{2+}$ 10 µM. **B.** The bar graph compares the mean inward current changed by glutamate alone with glutamate in the presence of Zn$^{2+}$ (10 µM). **C.** Glutamate (30 µM)-induced inward current was increased by the simultaneous application of Zn$^{2+}$ (300 nM). **D.** There is a significant difference between the means values created by glutamate alone and glutamate in the presence of Zn$^{2+}$ (300 nM) (* p < 0.05, ** p < 0.01).
is more sensitive to Zn$^{2+}$ inhibition than are the receptors consisted of γ subunits (Draguhn et al. 1990; Smart et al. 1991). In contrast, Zn$^{2+}$ was reported to potentiate GABA$_A$ receptor activity in the retinal Müller glial cells in some receptor subunits (Qian et al. 1996). In addition, in the rat hippocampus, the extracellular Zn$^{2+}$ also affected GABA$_\beta$ receptors in a biphasic manner by modulating GABA$_\beta$ binding biphasingly (Xie and Smart 1991). As can be seen from those studies, Zn$^{2+}$ has many effects on GABA.

Beyond effects on inhibitory neurotransmitters, glycine receptors, and GABA$_A$ receptors, Zn$^{2+}$ also has a powerful modulation effect on glutamate-mediated responses. Glutamate or excitatory amino-acid receptors are considered to be the main neurotransmitter receptors that modulate the fast synaptic excitation in the CNS of the mammal (Gasic and Hollmann 1992). It has long been known that a large amount of Zn$^{2+}$ is concentrated inside vesicles of the glutamatergic terminals in the CNS (Frederickson 1989; Choi and Koh 1998). This relation points toward the logical role of Zn$^{2+}$ in the modulation of glutamate response. Depending on the pharmacological functions and the interaction of characteristic agonists, the glutamate receptors are classified into many subtypes (Gasic and Hollmann 1992). Many different effects of Zn$^{2+}$ on different glutamate subtypes have been demonstrated. The presynaptic glutamate concentration released in the rat hippocampal CA1 and CA3, as well as the entorhinal cortex region, were attenuated by the perfusion with Zn$^{2+}$ (Takeda et al. 2003, 2004). At the single-channel level, Zn$^{2+}$ was proved to powerfully inhibit N-methyl-D-aspartate (NMDA) channel currents in murine neocortical neurons. Some main mechanisms explained for Zn$^{2+}$ inhibition of NMDA receptors includes the decrease in channel open frequency and the voltage-dependent amplitude reduction, which suggested a fast channel block (Christine and Choi 1990). Besides, some lines also show that Zn$^{2+}$ increased the excitation mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropriono acid (AMPA) receptors and NMDA receptors in mouse cultured cortical and rat cultured hippocampal CA1 neurons, respectively (Peters et al. 1987; Kim et al. 2002). To supplement the above abundant effects of Zn$^{2+}$, our study has proved that Zn$^{2+}$ also has biphasic effects on the glutamate-induced inward current in the SG neurons of Vc. The hypothesis for the opposite modulations of glutamatergic transmission by Zn$^{2+}$ is the different actions on different types of glutamate receptors which co-localized at the glutamatergic postsynapses (Rassendren et al. 1990). These mechanisms happen in a concentration dependence of Zn$^{2+}$ which corresponds with the difference in the apparent affinity values (300 nM for the potentiation and 10 µM for the inhibition). This specific characterization of Zn$^{2+}$ on glutamate receptors was also reported in the Xenopus oocytes that at a low concentration, Zn$^{2+}$ inhibited NMDA responses and increased non-NMDA response, but at higher concentration, Zn$^{2+}$ inhibited non-NMDA currents (Rassendren et al. 1990). Further investigation needs to be done to find out which types of glutamate receptor involving in the potentiation and inhibition phenomena between Zn$^{2+}$ and glutamate.

In conclusion, the above clear evidence reveals to some extent the diverse effects of Zn$^{2+}$ on different neurotransmitters in the SG neurons. The opposite influences of this metal ion may originate from its different processes that interact with various binding sites on different receptors and with distinct affinities. Some growing studies have elucidated that Zn$^{2+}$ also plays an important role in the modulation of pain transmission (Larson and Kitto 1997; Velazquez et al. 1999). Taken together, the regulatory action of Zn$^{2+}$ to the neurotransmitters in the SG neurons implies an important mechanism in pain information processing in the CNS which has a part in the plasticity of neuronal circuits. Further research needs to be done to discover the concrete mechanism by which Zn$^{2+}$ not only excites neurotransmitters but also inhibits receptors in the SG neurons of the Vc.

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Conflict of interest. The authors declare that they have no conflicts of interest.

References

Barberis A, Cherubini E, Mozrzymas JW (2000): Zinc inhibits miniature GABAergic currents by allosteric modulation of GABA$\alpha$ receptor gating. J. Neurosci. 20, 8618-8627.

Bloomenthal AB, Goldwater E, Pritchett DB, Harrison NL (1994): Biphasic modulation of the strychnine-sensitive glycine receptor by Zn$^{2+}$. Mol. Pharmacol. 46, 1156-1159.

Bowery NG, Smart TG (2006): GABA and glycine as neurotransmitters: a brief history. Br. J. Pharmacol. 147, S109-119.

Buhl EH, Otis TS, Mody I (1996): Zinc-induced collapse of augmented inhibition by GABA in a temporal lobe epilepsy model. Science 271, 369-373.

Burger PM, Hell J, Mehl E, Krasel C, Lottspeich F, Jahn R (1991): GABA and glycine in synaptic vesicles: storage and transport characteristics. Neuron 7, 287-293.

Choi DW, Yokoyama M, Koh J (1988): Zinc neurotoxicity in cortical cell culture. Neuroscience 24, 67-79.

Choi DW, Koh JY (1998): Zinc and brain injury. Annu. Rev. Neurosci. 21, 347-375.
Christensen H, Fonnum F (1991): Uptake of glycine, GABA and glutamate by synaptic vesicles isolated from different regions of rat CNS. Neurosci. Lett. 129, 217-220
https://doi.org/10.1016/0304-3908(91)90465-6

Christine CW, Choi DW (1990): Effect of zinc on NMDA receptor-mediated channel currents in cortical neurons. J. Neurosci. 10, 108-116
https://doi.org/10.1523/JNEUROSCI.10-01-00108.1990

Coleman JE (1992): Zinc proteins: enzymes, storage proteins, transcription factors, and replication proteins. Annu. Rev. Biochem. 61, 897-946
https://doi.org/10.1146/annurev.bi.61.070192.004341

Draguhn A, Verdorn TA, Ewert M, Seeburg PH, Sakmann B (1989): Zinc, a neurotoxin to cultured neurons, contaminates cytosolic flour prepared by traditional guananim methods. J. Neurosci. 12, 1523-1537
https://doi.org/10.1523/JNEUROSCI.12-04-01523.1992

Forbes II, Zalewski PD, Giannakis C (1991): Role for zinc in a cellular response mediated by protein kinase C in human B lymphocytes. Exp. Cell. Res. 195, 224-229
https://doi.org/10.1006/0014-4827(91)90521-U

Frederickson CJ, Kasarskis EJ, Ringo D, Frederickson RE (1987): A quinoline fluorescence method for visualizing and assaying the histochemically reactive zinc (boulton zinc) in the brain. J. Neurosci. Methods 20, 91-103
https://doi.org/10.1016/0165-0270(87)90042-2

Frederickson CJ (1989): Neurobiology of zinc and zinc-containing neurons. Int. Rev. Neurobiol. 31, 145-238
https://doi.org/10.1016/S0074-7742(08)60279-2

Frederickson CJ, Giblin LJ, Krezel A, McAdoo DJ, Mueller RN, Zeng Y, Balaji RV, Masalha R, Thompson RB, Fierke CA, et al. (2006): Concentrations of extracellular free zinc (pZn) in the central nervous system during simple anesthetization, ischemia and reperfusion. Exp. Neurol. 198, 285-293
https://doi.org/10.1016/j.expneurol.2005.08.030

Freund WD, Reddig S (1994): AMPA/Zn(2+)-induced neurotoxicity in rat primary cortical cultures: involvement of L-type calcium channels. Brain Res. 654, 257-264
https://doi.org/10.1016/0006-8993(94)90487-1

Gasic GP, Hollmann M (1992): Molecular neurobiology of glutamate receptors. Annu. Rev. Physiol. 54, 507-536
https://doi.org/10.1146/annurev.ph.54.030192.002451

Gower-Winter SD, Levenson CW (2012): Zinc in the central nervous system: From molecules to behavior. Biofactors 38, 186-193
https://doi.org/10.1002/biof.1012

Harrison NL, Gibbons SJ (1994): Zn2+: an endogenous modulator of ligand- and voltage-gated ion channels. Neuropharmacology 33, 935-952
https://doi.org/10.1016/0028-3908(94)90152-X

Hurley LS, Shraer RE (1972): Congenital malformations of the nervous system in zinc-deficient rats. In: Neurobiology of the Trace Metals Zinc and Copper (Ed. CC Pfeiffer), pp. 51-60, Academic Press, New York
https://doi.org/10.1016/B978-0-12-366851-6.50007-7

Karlin A, Akabas MH (1995): Toward a structural basis for the function of nicotinic acetylcholine receptors and their cousins. Neuron 15, 1231-1244
https://doi.org/10.1016/0896-6273(95)90004-7

Kay AR (2003): Evidence for chelatable zinc in the extracellular space of the hippocampus, but little evidence for synaptic release of Zn. J. Neurosci. 23, 6847-6855
https://doi.org/10.1523/JNEUROSCI.23-17-06847.2003

Kim TY, Hwang JJ, Yun SH, Jung MW, Koh JY (2002): Augmentation of zinc by NMDA receptor-mediated synaptic responses in CA1 of rat hippocampal slices: mediation by Src family tyrosine kinases. Synapse 46, 49-56
https://doi.org/10.1002/syn.10118

Kohno T, Kumamoto E, Higashi H, Shimoeji K, Yoshimura M (1999): Actions of opioids on excitatory and inhibitory transmission in substantia gelatinosa of adult rat spinal cord. J. Physiol. 518, 803-813
https://doi.org/10.1111/j.1469-7793.1999.0803p.x

Larson AA, Kitto KF (1997): Manipulations of zinc in the spinal cord, by intrathecal injection of zinc chloride, disodium-calcium-EDTA, or dipicolinic acid, alter nociceptive activity in mice. J. Pharmacol. Exp. Ther. 282, 1319-1325

Laube B, Kuhse J, Rundstrom N, Kirsch J, Schmieden V, Betz H (1995): Modulation of zinc ions of native rat and recombinant human inhibitory glycine receptors. J. Physiol. 483, 613-619
https://doi.org/10.1113/jphysiol.1995.sp020610

Laube B, Kuhse J, Betz H (2000): Kinetic and mutational analysis of Zn2+ modulation of recombinant human inhibitory glycine receptors. J. Physiol. 522, 215-230
https://doi.org/10.1111/j.1469-7793.2000.1-1-00215.x

Laube B (2002): Potentiation of inhibitory glycineergic neurotransmission by Zn2+: a synergistic interplay between presynaptic P2X2 and postsynaptic glycine receptors. Eur. J. Neurosci. 16, 1025-1036
https://doi.org/10.1046/j.1460-9568.2002.02170.x

Legendre P, Westbrook GL (1991): Noncompetitive inhibition of gamma-aminobutyric acidA channels by Zn. Mol. Pharmacol. 39, 267-274

Li P, Yang XL (1999): Zn2+ differentially modulates glycine receptors versus GABA receptors in isolated carp retinal third-order neurons. Neurosci. Lett. 269, 75-78
https://doi.org/10.1016/S0304-3909(99)00416-4

Light AR, Perl ER (1979): Spinal termination of functionally identified primary afferent fibers with slowly conducting myelinated fibers. J. Comp. Neurol. 186, 193-199
https://doi.org/10.1002/cne.9001860203

Lynch JW, Jacques P, Pierce KD, Schofield PR (1998): Zinc potentiation at strychnine-sensitive glycine receptors. J. Biol. Chem. 273, 10705-10711
https://doi.org/10.1074/jbc.M508303200

Lynch JW, Rajendra S, Pierce KD, Handford CA, Barry PH, Schofield PR (1998): Zinc potentiation of the glycine receptor chloride channel is mediated by Src family tyrosine kinases. EMBO J. 17, 1025-1036
https://doi.org/10.1093/emboj/17.1.110

Lynch JW, Rajendra S, Pierce KD, Handford CA, Barry PH, Schofield PR (1997): Identification of intracellular and extracellular domains mediating signal transduction in the inhibitory glycine receptor chloride channel. EMBO J. 16, 110-120
https://doi.org/10.1093/emboj/16.1.110

Miller PS, Da Silva HM, Smart TG (2005): Molecular basis for zinc potentiation at strychnine-sensitive glycine receptors. J. Biol. Chem. 280, 37877-37884
https://doi.org/10.1074/jbc.M508303200
Nguyen HT, Bhattrai JP, Park SJ, Lee JC, Cho DH, Han SK (2015): Enhanced GABA action on the substantia gelatinosa neurons of the medullary dorsal horn in the offspring of streptozotocin-injected mice. J. Diabetes Complications 29, 629-636 https://doi.org/10.1016/j.jdiacomp.2015.03.007

Peters S, Koh J, Choi DW (1987): Zinc selectively blocks the action of N-methyl-D-aspartate on cortical neurons. Science 236, 589-593 https://doi.org/10.1126/science.2883728

Pfeiffer F, Graham D, Betz H (1982): Purification by affinity chromatography of the glycine receptor of rat spinal cord. J. Biol. Chem. 257, 9389-9393

Price TJ, Cervero F, de Koninck Y (2005): Role of cation-chloridecotransporters (CCC) in pain and hyperalgesia. Curr. Top. Med. Chem. 5, 547-555 https://doi.org/10.2174/1568026054367629

Qian H, Malchow RP, Chappell RL, Ripps H (1996): Zinc enhances ionic currents induced in skate Muller (glial) cells by the inhibitory neurotransmitter GABA. Proc. Biol. Sci. 263, 791-796 https://doi.org/10.1098/rspb.1996.0118

Rassendren FA, Lory P, Pin JP, Nargeot J (1990): Zinc has opposite effects on NMDA and non-NMDA receptors expressed in Xenopus oocytes. Neuron 4, 733-740 https://doi.org/10.1016/0896-6273(90)90199-P

Ruiz A, Walker MC, Fabian-Fine R, Kullmann DM (2004): Endogenous zinc inhibits GABA(A) receptors in a hippocampal pathway. J. Neurophysiol. 91, 1091-1096 https://doi.org/10.1152/jn.00755.2003

Santos SF, Rebelo S, Derkach VA, Safarov BV (2007): Excitatory interneurons dominate sensory processing in the spinal substantia gelatinosa of rat. J. Physiol. 581, 241-254 https://doi.org/10.1113/jphysiol.2006.126912

Sivillotti L, Nistri A (1991): GABA receptor mechanisms in the central nervous system. Prog. Neurobiol. 36, 35-92 https://doi.org/10.1016/0301-0082(91)90036-Z

Smart TG, Moss SJ, Xie X, Huganir RL (1991): GABA(A) receptors are differentially sensitive to zinc: dependence on subunit composition. Br. J. Pharmacol. 103, 1837-1839 https://doi.org/10.1111/j.1476-5381.1991.tb12337.x

Smart TG (1992): A novel modulatory binding site for zinc on the GABAA receptor complex in cultured rat neurons. J. Physiol. 447, 587-625 https://doi.org/10.1113/jphysiol.1992.sp019020

Smart TG, Xie X, Krishke B (1994): Modulation of inhibitory and excitatory amino acid receptor ion channels by zinc. Prog. Neurobiol. 42, 393-441 https://doi.org/10.1016/0301-0082(94)90082-5

Takeda A, Minami A, Seki Y, Oku N (2003): Inhibitory function of zinc against excitation of hippocampal glutamatergic neurons. Epilepsy Res. 57, 169-174 https://doi.org/10.1016/j.epilepsyres.2003.11.003

Takeda A, Minami A, Seki Y, Oku N (2004): Differential effects of zinc on glutamatergic and GABAergic neurotransmitter systems in the hippocampus. J. Neurosci. Res. 75, 225-229 https://doi.org/10.1002/jnr.10846

Thompson RB, Whtsell WO Jr, Malwal BP, Fierce KA, Frederickson CJ (2000): Fluorescence microscopy of stimulated Zn(II) release from organotypic cultures of mammalian hippocampus using a carbonic anhydrase-based biosensor system. J. Neurosci. Methods 96, 35-45 https://doi.org/10.1016/S0165-0270(99)00183-1

Todd AJ, Watt C, Spike RC, Sieghart W (1996): Colocalization of GABA, glycine, and their receptors at synapses in the rat spinal cord. J. Neurosci. 16, 974-982 https://doi.org/10.1523/JNEUROSCI.16-03-00974.1996

Todd AJ (2002): Anatomy of primary afferents and projection neurons in the rat spinal dorsal horn with particular emphasis on substance P and the neurokinin 1 receptor. Exp. Physiol. 87, 245-249 https://doi.org/10.1113/eph8720351

Vallee BL, Falchuk KH (1981): Zinc and gene expression. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 294, 185-197 https://doi.org/10.1098/rstb.1981.0098

Velazquez RA, Cai Y, Shi Q, Larson AA (1999): The distribution of zinc selenite and expression of metallothionein-III mRNA in the spinal cord and dorsal root ganglia of the rat suggest a role for zinc in sensory transmission. J. Neurosci. 19, 2288-2300 https://doi.org/10.1523/JNEUROSCI.19-06-02288.1999

Verdoorn TA, Draguhn A, Ymer S, Seeburg PH, Sakmann B (1990): Functional properties of recombinant rat GABAA receptors depend upon subunit composition. Neuron 4, 919-928 https://doi.org/10.1016/0896-6273(90)90145-6

Weinberger RP, Rostas JA (1991): Effect of zinc on calmodulin-stimulated protein kinase II and protein phosphorylation in rat cerebral cortex. J. Neurochem. 57, 605-614 https://doi.org/10.1111/j.1471-4159.1991.tb03791.x

Weiss JH, Hartley DM, Koh JY, Choi DW (1993): AMPA receptor activation potentiates zinc neurotoxicity. Neuron 10, 43-49 https://doi.org/10.1016/0896-6273(93)90240-R

Winegar BD, Lansman JB (1990): Voltage-dependent block by zinc of single calcium channels in mouse myotubes. J. Physiol. 425, 563-578 https://doi.org/10.1113/physiol.1990.sp018118

Xie XM, Smart TG (1991): A physiological role for endogenous zinc in rat hippocampal synaptic neurotransmission. Nature 349, 521-524 https://doi.org/10.1038/349521a0

Xie XM, Smart TG (1993): Giant GABAB-mediated synaptic potentials induced by zinc in the rat hippocampus: paradoxical effects of zinc on the GABAB receptor. Eur. J. Neurosci. 5, 430-436 https://doi.org/10.1111/j.1460-9588.1993.tb00509.x

Yin HZ, Weiss JH (1995): Zn(2+) permeates Ca(2+) permeable AMPA/kainate channels and triggers selective neural injury. Neuroreport 6, 2553-2556 https://doi.org/10.1097/00001756-199512150-00025

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