Suppression by Zinc of AMPA Receptor-Mediated Synaptic Transmission in the Retina

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Zhang, Dao-Qi, Christophe Ribelayga, Stuart C. Mangel, and Douglas G. McMahon. Suppression by zinc of AMPA receptor-mediated synaptic transmission in the retina. J Neurophysiol 88: 1245–1251, 2002; 10.1152/jn.00008.2002. Zinc is strikingly co-localized with glutamate-containing vesicles in the synaptic terminals of retinal photoreceptors, and it is thought to be co-released with glutamate onto postsynaptic neurons such as horizontal cells and bipolar cells. Here we examined exogenous zinc modulation of glutamate receptors on cultured retinal horizontal cells using patch-clamp recording and endogenous zinc effect on intact horizontal cells using intracellular recording techniques. Application of 3, 30, and 300 μM zinc reduced the whole cell peak current response to 200 μM glutamate by 2, 30, and 56%, respectively. Zinc suppression of glutamate response persisted in the presence of 10 μM cyclothiazide (CTZ). Glutamate responses of outside-out patches were completely abolished by 30 μM 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine (GYKI 52466), and the receptor desensitization was blocked by 30 μM CTZ, indicating that receptor target for the zinc action on horizontal cells is α-amino-3-hydroxy-5-methyl-4-isoxazoleproponic acid (AMPA) receptors. Zinc decreased the amplitude of outside-out patch peak current without an effect on either its 10–90% rise time or the rate of receptor desensitization. Dose-response curves for glutamate show that zinc reduced the maximal current evoked by glutamate and increased EC50 from 50 ± 3 to 70 ± 6 μM without changing the Hill coefficient. Chelation of endogenous zinc with 1 mM Ca-EDTA depolarized horizontal cells in the intact retina by 3 mV, consistent with relief of the partial glutamate receptor inhibition by zinc. Overall, the results describe a unimodal form of zinc modulation of AMPA-type glutamate receptor responses not previously described in native neuronal preparations and a novel role for endogenous zinc in modulating neurotransmission.

INTRODUCTION

The trace metal zinc is a potential endogenous neuromodulator in the vertebrate retina. Zinc is concentrated in the terminals of photoreceptors in fish and tiger salamander retina (Qian et al. 1997; Wu et al. 1993) and distributed throughout the mammal retina including the two synaptic plexiform layers (Akagi et al. 2001; Ugarte and Osborne 1998). At the ultrastructural level, endogenous zinc is colocalized with glutamatergic synaptic vesicles in neural processes of the outer plexiform layer and inner plexiform layer (Akagi et al. 2001). In addition, the distribution of zinc in rat photoreceptors also varies during light and dark adaptation (Ugarte and Osborne 1999). Thus it has been suggested that free zinc is released from synaptic terminals into synaptic clefts during visual signaling (Akagi et al. 2001). Although the exact concentration of zinc in retinal synaptic clefts is unclear, zinc concentrations as high as 300 μM can be obtained in glutamatergic synaptic clefts with intense activity in the hippocampus (Assaf and Chung 1984). Given the possibility that zinc is co-released from photoreceptors, zinc may play important physiological roles in modulating the postsynaptic activity of membrane receptors and ion channels.

Horizontal cells are second order retinal interneurons that receive excitatory synaptic input via glutamatergic synapses from photoreceptors. They also provide inhibitory feedback via GABAergic synapses to photoreceptors. In the dark, glutamate is tonically released from photoreceptors and activates glutamate receptors to depolarize horizontal cells. Several lines of evidence have shown that α-amino-3-hydroxy-5-methyl-4-isoxazoleproponic acid (AMPA)-type glutamate receptors are expressed on retinal horizontal cells and play a primary role in mediating signal transmission in the outer retina (Blanco and de la Villa 1999; Eliasof and Jahr 1997; Ishida and Neyton 1985; Knapp and Dowling 1987; Lu et al. 1998; Yang et al. 1998).

In this study, we examined the interaction of zinc and glutamate on horizontal cell responses in dispersed cells and in the intact retina. We found that isolated horizontal cells exhibit AMPA receptor currents that are partially inhibited by zinc beginning at micromolar concentrations. Ca-EDTA, a zinc chelator, relieved zinc suppression of glutamate receptors on isolated cells and depolarized horizontal cells in the intact retina. These novel findings provide evidence that zinc may modulate the responsiveness of glutamatergic neurons in the retina and elsewhere in the CNS. Part of this work has been summarized in a review chapter (McMahon et al. 2001).

METHODS

Preparation

Dark-adapted adult hybrid striped bass (Roccus chrysops × R. saxitilis) were killed in accordance with National Institutes of Health guidelines for animal use. For cell culture, retinas were removed under dim red light and then incubated in L-15 media (GIBCO BRL,IRECTION
Rockville, MD) containing 20 U/ml papain (Worthington Biochemical Corp., Lakewood, NJ) activated with cysteine and EDTA. The retinas were incubated in the L15/papain solution for 40 min followed by six changes of fresh 1-L 15 media, and dissociated by repeated passage through a serological pipette. Isolated cells were plated on plastic 35-mm dishes containing fresh L-15 medium. Cultures were maintained at 17°C and the cells were used following 1–4 days in culture. Horizontal cell subtypes H1–H4 can be easily identified by their morphology in culture (Dowling et al. 1985). For intact retina recording, isolated retinas were transferred to a perfusion chamber and stabilized with a nylon mesh. Retinas were dark-adapted for 30 min before experiment.

Solutions

The patch-clamp recording extracellular solution was (in mM) 145 NaCl, 2.5 KCl, 0.5 MgCl₂, 0.5 MgSO₄, 2.5 CaCl₂, 2 NaHCO₃, 10 HEPES, 10 glucose, and 1 mg/ml BSA; pH was adjusted to 7.5 with NaOH. Pipette solution contained the following (in mM): 72 K-glucuronate, 48 KF (potassium fluoride or 48 mM K-glucuronate), 4 KCl, 1 CaCl₂, 11 EGTA, 11 HEPES, 1 MgATP, and 0.1 NaGTP; pH was adjusted to 7.5 with KOH. The presence of KF did not affect zinc action on glutamate receptors. The extracellular solutions were similar for outside-out patch recordings to the above except for the addition of ZnCl₂. 1% stock solution (100 mM) of ZnCl₂ was purchased from Sigma (St. Louis, MO). (2)

Macroscopic currents were measured by the whole cell patch-clamp technique. Patch pipettes (5–10 MΩ) were fabricated from Corning 7052 glass (AM Systems Inc., Carlsborg, WA) and filled with the pipette solution described above. Whole cell currents were recorded using an Axopatch 1-D amplifier (Axon Instruments Inc.) in voltage-clamp mode. Voltage commands and data acquisition were performed using pClamp 8 software (Axon Instruments Inc.). Patch currents were recorded from outside-out patches using fire-polished glass pipettes coated with Sylgard.

Intracellular recording

Electrodes were made with borosilicate glass (World Precision Instruments, Inc.; OD, 1.2 mm; ID, 0.68 mm) and pulled with a Flaming-Brown puller (Sutter Instruments Co., Novato, CA). They were filled with 2 M KCl (R = 150–200 MΩ). Retinas were stimulated (500-ms duration every 8 s) with a dim red light (650 nm; intensity: −1 logIₒ, where Iₒ = 2.35 μW/cm²). Intensity-response series using 500- and 650-nm stimuli were also obtained. The maximum intensity of the 500-nm light was 1.24 μW/cm². All light-evoked data are from hybrid striped bass L-type cone horizontal cells, identified by their chromatic characteristics.

Data analysis

Glutamate dose-response curves were fit with the Hill equation

\[ I \_{\text{max}} = I \_{\text{max}} \cdot A^n/(EC_{50} + A^n) \]

where \( I \_{\text{max}} \) is the cell membrane current elicited by a given agonist concentration (A), \( EC_{50} \) is the agonist concentration that elicits a half-maximal response, \( I \_{\text{max}} \) is the measured maximal response, and n is the Hill coefficient; the normalized percent inhibition of zinc was calculated by the equation: \( (I \_{\text{control}} - I \_{\text{zinc}})/(I \_{\text{control}}) \times 100\% \).

The data are presented as mean ± SE. P values were calculated using the paired t-test.

RESULTS

AMPA-type glutamate receptors on horizontal cells

To elucidate the glutamate receptor subtypes present on bass horizontal cells, pharmacological experiments were performed on outside-out macropatches excised from isolated horizontal cells. A high concentration of glutamate (3 mM) and the maximal exchange speed of the perfusion system were used to obtain full patch-current activation and desensitization. Figure 1A (left) shows a typical inward patch current induced by 3 mM glutamate that rose rapidly to a peak and then desensitized to a steady-state level. In 12 patches, the average of peak current was 13.7 ± 7.7 pA with a 10–90% rise time of 0.52 ± 0.02 ms. The decay of receptor desensitization was fit with one exponential with a time constant of 1.11 ± 0.25 ms (n = 12). These results are consistent with previously published studies on catfish and perch horizontal cells (Eliazof and Jahr 1997; Schmidt et al. 1994). To identify glutamate receptor subtypes, we first examined the effect of the specific AMPA receptor antagonist, GYKI-52466 (Donevan and Rogawski 1993). As illustrated in Fig. 1A (middle), 30 μM GYKI-52466 almost completely abolished the glutamate-induced current. Similar results were obtained in four other outside-out patches. Whereas the activation of glutamate currents was blocked by an AMPA receptor antagonist, the desensitization of a patch current elicited by glutamate was almost completely inhibited by 30 μM CTZ (Fig. 1B), a specific blocker of AMPA receptor desensitization (Partin et al. 1993). The results were consistent in four additional outside-out patches. In addition, five other outside-out patches that were activated by glutamate were
**Inhibition by zinc of horizontal cell AMPA receptors**

We next tested the effects of various zinc concentrations on currents evoked by glutamate in cultured bass retinal horizontal cells. Figure 2A shows partial suppression by zinc of sustained responses to 200 μM glutamate in an H2 horizontal cell. Zinc (3 μM) did not change the glutamate response (95 vs. 94 pA), whereas 30 and 300 μM zinc significantly decreased the current from 95 to 70 pA and 47 pA, respectively. Similar results were obtained from H1-type horizontal cells and H4-type horizontal cells (data not shown). In five H2-type horizontal cells, glutamate-induced currents remained unchanged by 3 μM zinc (a reduction of 5 ± 2%, *P* > 0.05), but both 30 and 300 μM zinc significantly decreased the current by 30 ± 3% (*P* < 0.05) and 56 ± 5% (*P* < 0.001), respectively (Fig. 2B). Recovery from zinc inhibition occurred rapidly on washout. Application of zinc alone did not evoke currents. Zinc suppression of glutamate responses persisted in the presence of 10 μM CTZ (Fig. 2C). As above, application of 30 and 300 μM zinc reduced the current by 25 ± 3% (*P* < 0.05) and by 64 ± 2% (*P* < 0.001), respectively, whereas a lower concentration of zinc (3 μM) was ineffective (*P* > 0.05; *n* = 5, Fig. 2D). Similar results were obtained by using AMPA and kainate as glutamate receptor agonists (data not shown).

Ca-EDTA, which is a potent chelator of zinc (Li et al. 2001; Westergaard et al. 1995), relieved zinc inhibition of glutamate receptors. In the presence of 1 mM Ca-EDTA, the inhibition by 300 μM zinc of currents induced by 200 μM glutamate plus 10 μM CTZ was reduced from 59 ± 1.7 to 19 ± 1.1% (*P* < 0.001, *n* = 4). Since Ca-EDTA alone reduced the response by 18 ± 1.2%, this suggests that 1 mM Ca-EDTA completely relieved zinc inhibition of glutamate receptors and had a slight inhibitory effect of its own on glutamate responses.

**Zinc does not affect AMPA receptor desensitization kinetics**

To investigate whether zinc affects the rate of receptor desensitization, we tested the effects of zinc on outside-out macropatch currents induced by high concentrations of glutamate-induced inward current with a strong desensitization in an outside-out macro- 

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**Figure 1.** α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors expressed on cultured bass horizontal cells. A: 3 mM glutamate induced inward current with a strong desensitization in an outside-out macropatch excised from an H2-type horizontal cell (left). This current was blocked by 30 μM 1-(4-aminophenyl)-2-methyl-7,8-methylenedioxy-5H-1,2,3-benzodiazepine (GYKI 52466) (middle) and recovered on washout (right). B: desensitizing current induced by 3 mM glutamate (left) in an outside-out patch was inhibited by 30 μM cyclothiazide (CTZ) (middle) and recovered on washout (right). C: in an outside-out patch in which 3 mM glutamate evoked inward current (left), 10 μM (2S,4R)-4-methylglutamate (SYM 2841) (middle) and 10 μM (RS)-2-amino-3-(5-tert-butyl-3-hydroxy-4-isothiazolyl)propionic acid (ATPA) (right) failed to produce the inward current. Each trace is averages of 3 consecutive responses. Pipette potential was held at −60 mV.

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**Figure 2.** Inhibition of glutamate-activated currents of horizontal cells by external zinc. A: traces are the inward currents evoked by 200 μM glutamate in the absence and presence of 3, 30, and 300 μM zinc in an H2-type horizontal cell (*V*<sub>1H</sub> = −60 mV). B: normalized zinc percent inhibition of currents induced by 200 μM glutamate (*P* < 0.05; *n* = 5). C: traces are the inward currents evoked by 300 μM glutamate plus 10 μM CTZ in the absence and presence of 3, 30, and 300 μM zinc in an H2-type horizontal cell. D: normalized zinc percent inhibition of currents induced by 200 μM glutamate plus 10 μM CTZ (*P* < 0.05; *n* = 5).
mate applied by ultrafast solution switching. Typical current responses and partial suppression by zinc are shown in Fig. 3A. On average, peak current was decreased 32% from 12.5 ± 4.1 to 8.4 ± 2.9 pA \((P < 0.05)\) in the presence of 30 \(\mu M\) zinc and 55% from 12.5 ± 4.1 to 5.6 ± 1.8 pA \((P < 0.001)\) in the presence of 300 \(\mu M\) zinc \((n = 5)\). Normalized traces in Fig. 3B show that zinc did not change the 10–90% rising time or the rate of receptor desensitization. The 10–90% rise time of inward currents \((0.5 ± 0.04 \text{ ms})\) in the control cells remained unchanged in the presence of either 30 \(\mu M\) \((0.49 ± 0.04 \text{ ms}, P > 0.05, n = 5)\) or 300 \(\mu M\) zinc \((0.53 ± 0.02 \text{ ms}, P > 0.05, n = 5, \text{Fig. 3C})\). Meanwhile, neither 30 \(\mu M\) zinc nor 300 \(\mu M\) zinc changed the time constant of desensitization \((1.05 ± 0.05 \text{ ms})\) in the control; 1.09 ± 0.07 ms in the presence of 30 \(\mu M\) zinc and 1.11 ± 0.09 ms in the presence of 300 \(\mu M\) zinc, \(n = 5, \text{Fig. 3C})\).

Zinc reduces the receptor efficacy and affinity for glutamate

To explore whether zinc modulates the affinity and efficacy of AMPA receptors for glutamate, we examined the glutamate dose-response relationship in the absence and presence of zinc. Zinc decreased the apparent affinity of receptors for glutamate, shifting the half-maximal effective concentration, \(EC_{50}\), rightward, from 50 ± 3 to 70 ± 6 \(\mu M\) in the presence of 200 \(\mu M\) zinc without changing the Hill coefficient \((2.2 ± 0.2 \text{ vs. } 2.0 ± 0.3, \text{Fig. 4A})\). Zinc also reduced the efficacy of glutamate as illustrated by a decrease in the maximal current, \(I_{\text{max}}\), from 100 ± 20 to 64 ± 2 pA. The effect of zinc on the glutamate dose-response function was similar in the presence of CTZ. As illustrated in Fig. 4B, \(I_{\text{max}}\) was decreased 44% by 200 \(\mu M\) zinc \((2532 ± 64 \text{ vs. } 1434 ± 6 \text{ pA})\). Moreover, the curve was shifted rightward with an increase in \(EC_{50}\), from 63 ± 2 \(\mu M\) in the absence of zinc to 91 ± 1 \(\mu M\) in the presence of zinc.
The inhibition and receptor subunit composition of zinc containing sites are targets for zinc action. Horizontal cell glutamate receptors, directly postsynaptic to the retinal zinc in the photoreceptor terminals, it is sensible that 1998; Wu et al. 1993). With the primary concentration of hyperpolarization of the horizontal cell membrane potential.

**DISCUSSION**

The key results of this study are that exogenous zinc partially inhibits the AMPA receptor responses of retinal horizontal cells and that endogenous retinal zinc modulates neurotransmission to horizontal cells in the intact retina, resulting in hyperpolarization of the horizontal cell membrane potential. This adds to the potential neuromodulatory roles for this metal ion, which also has complex effects on retinal GABA receptors, neurotransmitter transporters and transmitter release (Dong and Werblin 1995; Qian et al. 1997; Spiridon et al. 1998; Wu et al. 1993). With the primary concentration of retinal zinc in the photoreceptor terminals, it is sensible that horizontal cell glutamate receptors, directly postsynaptic to the zinc containing sites, are targets for zinc action.

**Zinc inhibition and receptor subunit composition**

A unique aspect of our findings is that zinc reduced AMPA receptor responses in a monotonic dose-dependent manner. However, it was effective in both whole cell and excised outside-out patch recordings, a cytoplasmic second messenger is unlikely to mediate zinc inhibition of AMPA receptors. Therefore, zinc binding site for inhibition seems most likely to be exposed on the extracellular aspect of receptor, similar to sites known to modulate NMDA and GABA receptors (Choi and ...
have observed (IC_{50} for glutamate or glutamate plus CTZ were increased in the presence of receptor desensitization blockade, and the rate of calcium entry decreased, indicating zinc does not inhibit AMPA receptor response by enhancing receptor desensitization and zinc is unlikely to bind at the CTZ-binding site. Zinc decreases the efficacy of glutamate on AMPA receptors. This effect of zinc is opposite to the actions of nitric oxide and dopamine, two endogenous modulators of glutamate receptors in the retina, which have been shown to enhance the maximum glutamate current (Kruse and Schmidt 1993; McMahon and Schmidt 1999). In addition, the EC_{50} for glutamate or glutamate plus CTZ were increased in the presence of zinc, indicating an element of competitive inhibition at the agonist-recognition site was present and zinc may also reduce the potency of glutamate on AMPA receptors.

The zinc-binding site is relatively low affinity because the concentration of zinc necessary to inhibit AMPA receptors is higher than that needed to modulate NMDA receptors and GABA receptors in retinal neurons (Qian et al. 1997; Westbrook and Mayer 1987). However, whereas the concentration of free zinc in horizontal cell synaptic clefts is unknown, in the hippocampus, synaptic zinc concentrations can reach 200–300 μM (Assaf and Chung 1984), suggesting that the effects we have observed (IC_{50} = 168 μM) are indeed physiologically relevant (McMahon et al. 2001).

Endogenous zinc modulates glutamatergic transmission in the retina

The zinc inhibition of AMPA receptor-mediated glutamate responses that we have described in dissociated retinal neurons could significantly modulate glutamatergic transmission in the retina, and by implication, other regions of the CNS. Multiple sites for zinc action have already been identified in the outer retina. These individual mechanisms could each result in horizontal cell membrane depolarization or hyperpolarization through relief of different zinc actions in the intact retina on zinc chelation. Among those mechanisms are relief of horizontal cell AMPA receptor inhibition and Ca^{2+} current blockade in photoreceptor terminals, which could produce horizontal cell membrane depolarization. Other possible contributing mechanisms including relief of zinc inhibition of GABA feedback to photoreceptors and glutamate transporters that would evoke cell membrane hyperpolarization (Spiridon et al. 1998; Wu et al. 1993). Additionally, the direct effect of Ca-EDTA on AMPA receptors shown in cultured horizontal cells could also induce horizontal cell membrane hyperpolarization. Our zinc chelation results show depolarization of cell dark membrane potential evoked by Ca-EDTA, which is a sum of horizontal cell dark membrane potential changes through relief of the above zinc action sites. Therefore it is reasonable to conclude that the effect of chelation to zinc effects on AMPA receptors, at least partially, contributes to the depolarization of horizontal cell dark membrane potential. A recent report using skate retina also found that chelation of endogenous zinc increased inward current in horizontal cells, consistent with the glutamate receptor modulation we have reported and/or increased transmitter release from photoreceptors (Chapelle and Redenti 2001). Thus modulation of AMPA receptors by zinc is one of a range of physiological mechanisms by which synaptically released zinc affects the function of retinal neurons. Zinc may also play an important role in certain pathological conditions; for example, during retinal ischemia, glutamate and zinc are released from photoreceptors, and retinal glutamate and zinc levels may, as a result, be elevated. In this case, zinc’s reduction of the responsiveness of retinal neurons to glutamate may protect them from excitotoxic neurodegeneration (Ugarte and Osborne 1999).

REFERENCES


Zn$^{2+}$ MODULATION OF RETINAL AMPA RECEPTORS


