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

DAO-QI ZHANG,[1] CHRISTOPHE RIBELAYGA,[2] STUART C. MANGEL,[2] AND DOUGLAS G. McMAHON[1]
[1]*Department of Physiology, University of Kentucky, Lexington, Kentucky 40536-0084; and* [2]*Department of Neurobiology, University of Alabama School of Medicine, Birmingham, Alabama 35294-0021*

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 of 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 EC_{50} from 50 $\pm$ 3 to 70 $\pm$ 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 with glutamate 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 glutamoceptive 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. saxitalis*) 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,

---

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 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 retinae were transferred to a perfusion chamber and stabilized with a nylon mesh. Retinae 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 MgCl$_2$, 0.5 MgSO$_4$, 2.5 CaCl$_2$, 2 NaHCO$_3$, 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-gluconate, 48 KF (potassium fluoride or 48 mM K-gluconate), 4 KCl, 1 CaCl$_2$, 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 10 mM 4-AP (4-aminopyridine) and 10 mM tetraethylammonium (TEA). The intracellular pipette solution contained (in mM) 140 CsCl, 4 NaCl, 0.5 CaCl$_2$, 5 EGTA, 10 TEA, 1 MgATP, 0.1 NaGTP, and 10 HEPES, buffered to pH 7.5 with CsOH (Lasater 1990). Composition of the Ringer for superfused retinas was as follows (in mM): 110 NaCl, 30 Na-bicarbonate, 1 CaCl$_2$, 20 glucose, 2.5 KCl, and 1 MgCl$_2$, bubbled with 95%O$_2$-5% CO$_2$; pH was measured at 7.4. AMPA, cyclothiazide (CTZ), glutamate, 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine (GYKI 52466), kainate, EDTA, and ZnCl$_2$ were purchased from Sigma (St. Louis, MO). (2S,4R)-4-methylglutamate (SYM 2841) and (RS)-2-amino-3-(5-tert-butyl-3-hydroxy-4-isothiazolyl)propionic acid (ATPA) were purchased from Tocris Cookson (Ballwin, MO). All pharmacological agents were prepared fresh daily. Glutamate, kainate, and ZnCl$_2$ were simply dissolved in the working solution. AMPA, CTZ, and GYKI-52466 were first dissolved in dimethyl sulfoxide (DMSO, <0.01% in working solution), and SYM-2081 and ATPA were dissolved in NaOH as a stock solution (100 mM).

Ultrafast solution application

Ultrafast solution exchange was achieved using a θ tube mounted on a LSS 3100 piezoelectric driven actuator (Burleigh Instruments, Inc., Fishers, NY). The θ glass tubing (TGC 200–10; Warner Instrument Corp., Hamden, CT) was pulled to an outer tip diameter of 100–150 μm. To minimize the dead space along the application pipette, microfilament tubing MF 28G (World Precision Instruments, Inc., Sarasota, FL) was inserted up to the tip, and the θ tubing was filled with molten wax almost to the tip. The movement of a θ tube was controlled by a voltage waveform input from Clampex 8 software (Axon Instruments Inc., Foster City, CA) to the piezoelectric driver that consisted of ramps from 0 to 4, 4 to 6, and 6 to 10 mV. For whole cell recording, durations of the ramp waveform were 40, 20, and 40 ms, respectively. In this case, the 10–90% exchange of solution around the open tip of a pipette occurred within 40 ± 0.7 ms (n = 5). For patch-current recording, the durations of ramp waveform were 4, 1, and 4 ms, respectively, and 10–90% rise times were 0.38 ± 0.08 ms (n = 6).

Patch-clamp recording

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 logI_o where I_o = 2.35 μW/cm^2). Intensity-response series using 500- and 650-nm stimuli were also obtained. The maximum intensity of the 500-nm light was 1.24 μW/cm^2. 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

$$I_{mem} = I_{max} \cdot A^n / (EC_{50}^n + A^n)$$

where I_{mem} 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_{max} is the measured maximal response, and n is the Hill coefficient; the normalized percent inhibition of zinc was calculated by the equation: $(I_{control} - I_{Zn})/I_{control} \times 100\%$.

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 (Eliasof 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
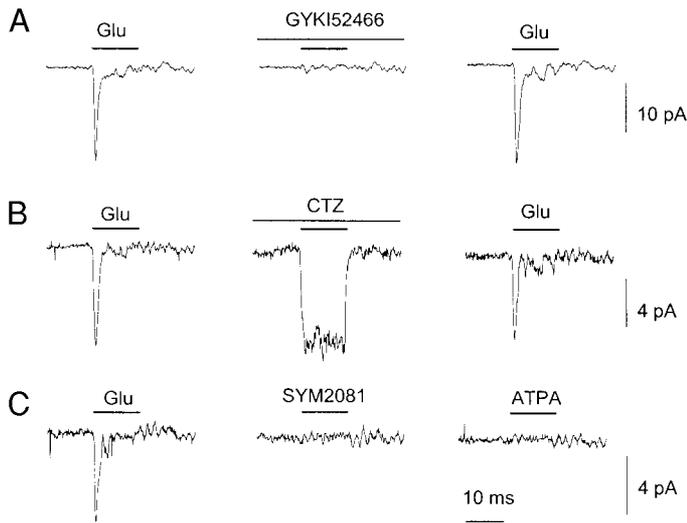
FIG. 1. α-Amino-3-hydroxy-5-methyl-4-isoxazoleproponic acid (AMPA) receptors expressed on cultured bass horizontal cells. *A*: 3 mM glutamate induced inward current with a strong desensitization in an outside-out macropatch excised from an H2-type horizontal cell (*left*). This current was blocked by 30 μM 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-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 (2*S*,4*R*)-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.

insensitive to the selective GluR6 agonist SYM2081 (Zhou et al. 1997) and the selective GluR5 agonist ATPA (Cui and Mayer 1999). Figure 1*C* illustrates an example that neither 10 μM SYM2081 (*middle*) nor 10 μM ATPA (*right*) produced the inward current on an outside-out micropatch. Taken together, the overall results suggest that cultured bass horizontal cells express glutamate receptors which are AMPA-type, with no or minimal contribution of NMDA or kainate-type receptors.

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 2*A* 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. 2*B*). 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. 2*C*). 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. 2*D*). 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 gluta-
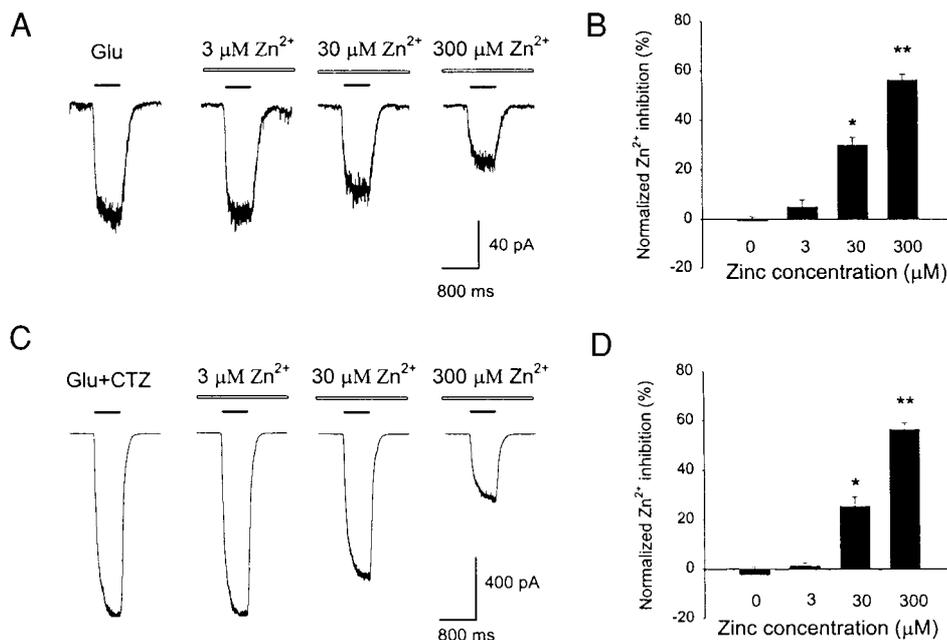

FIG. 2. Inhibition of glutamate-activated currents of horizontal cells by external zinc. *A*: traces are the inward currents evoked by 200 μM glutamate in the absence and presence of 3, 30, and 300 μM zinc in an H2-type horizontal cell ($V_H = -60$ mV). *B*: normalized zinc percent inhibition of currents induced by 200 μM glutamate (*$P < 0.05$, **$P < 0.001$; $n = 5$). *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 (*$P < 0.05$, **$P < 0.001$; $n = 5$).
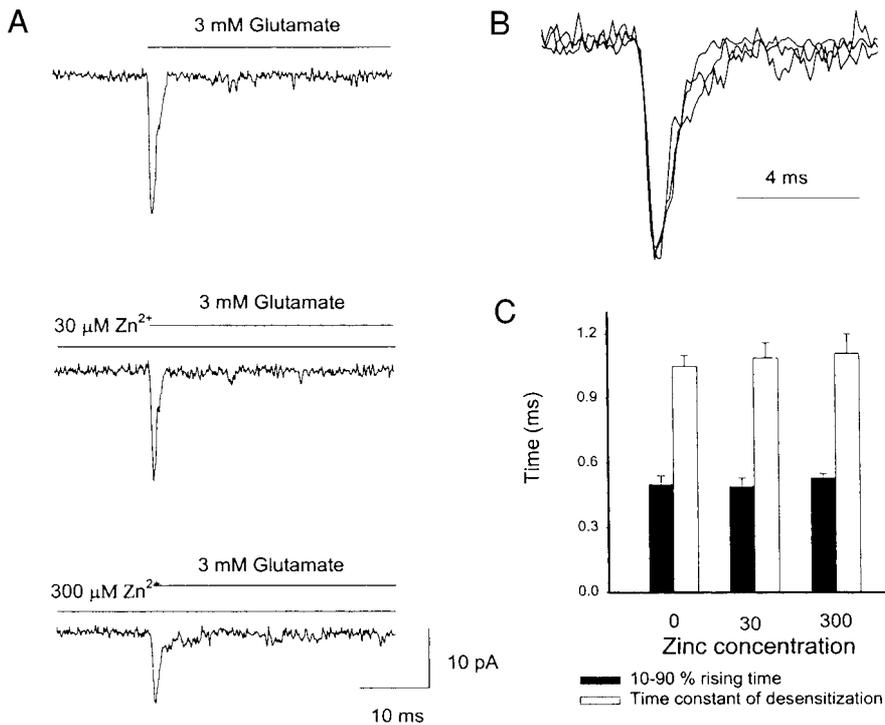
FIG. 3. Zinc inhibition of AMPA receptor currents in an outside-out macropatch excised from an H2-type horizontal cell. *A*: 3 mM glutamate induced current is shown in *top trace*. Current was reduced by 30 μM zinc (*middle trace*) and 300 μM zinc (*bottom trace*). *B*: normalized traces in *A* show zinc did not change the 10–90% rising time or the rate of receptor desensitization. Each trace is an average of 3 consecutive responses. Pipette potential was held at -60 mV. *C*: average of zinc effects on the 10–90% rise time and the rate of receptor desensitization ($n = 5$).

mate applied by ultrafast solution switching. Typical current responses and partial suppression by zinc are shown in Fig. 3*A*. On average, peak current was decreased 32% from 12.5 $\pm$ 4.1 to 8.4 $\pm$ 2.9 pA ($P < 0.05$) in the presence of 30 μM zinc and 55% from 12.5 $\pm$ 4.1 to 5.6 $\pm$ 1.8 pA ($P < 0.001$) in the presence of 300 μM zinc ($n = 5$). Normalized traces in Fig. 3*B* 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 $\pm$ 0.04 ms) in the control cells remained unchanged in the presence of either 30 μM (0.49 $\pm$ 0.04 ms, $P > 0.05$, $n = 5$) or 300 μM zinc (0.53 $\pm$ 0.02 ms, $P > 0.05$, $n = 5$, Fig. 3*C*). Meanwhile, neither 30 μM zinc nor 300 μM zinc changed the time constant of desensitization (1.05 $\pm$ 0.05 ms in the control; 1.09 $\pm$ 0.07 ms in the presence of 30 μM zinc and 1.11 $\pm$ 0.09 ms in the presence of 300 μM zinc, $n = 5$, Fig. 3*C*).

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 $\pm$ 3 to 70 $\pm$ 6 μM in the presence of 200 μM zinc without changing the Hill coefficient (2.2 $\pm$ 0.2 vs. 2.0 $\pm$ 0.3, Fig. 4*A*). Zinc also reduced the efficacy of glutamate as illustrated by a decrease in the maximal current, I_{max}, from 100 $\pm$ 2 to 64 $\pm$ 2 pA. The effect of zinc on the glutamate dose-response function was similar in the presence of CTZ. As illustrated in Fig. 4*B*, I_{max} was decreased 44% by 200 μM zinc (2532 $\pm$ 64 vs. 1434 $\pm$ 6 pA). Moreover, the curve was shifted rightward with an increase in EC_{50} from 63 $\pm$ 2 μM in the absence of zinc to 91 $\pm$ 1 μM in the presence of zinc,
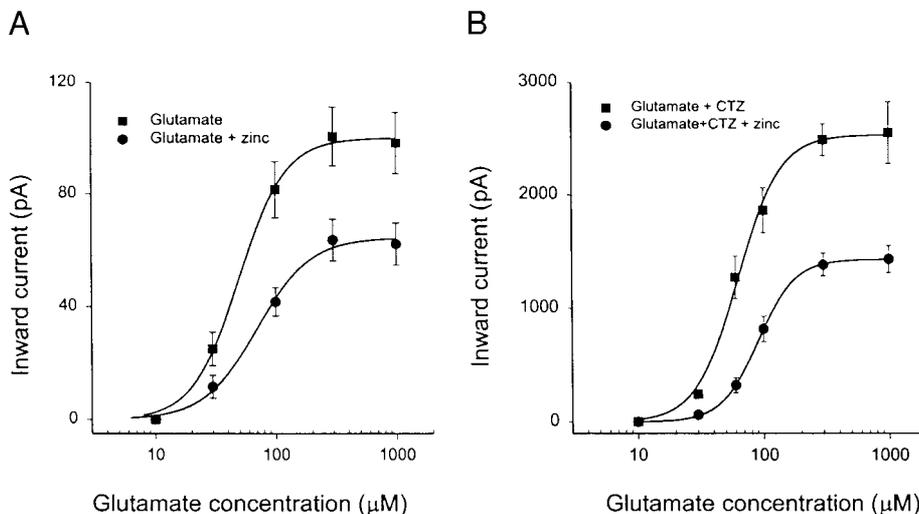

FIG. 4. Zinc reduced the receptor affinity for glutamate and the maximal current. *A*: concentration-response curves for glutamate in the absence (■) and presence (●) of 200 μM zinc. *B*: concentration-response relationships for glutamate plus CTZ (30 μM) in the absence (■) and presence of zinc (●). Each point is the mean of data from 4–6 cells. The smooth curves were fit by the Hill equation.
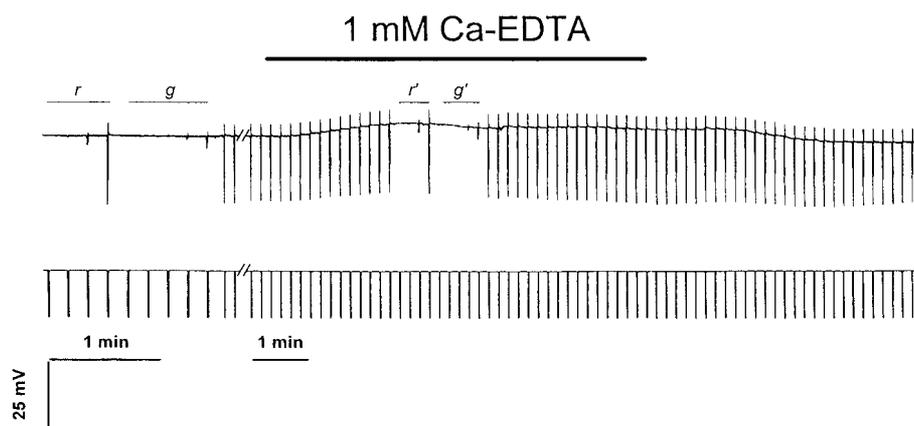
1 mM Ca-EDTA

FIG. 5. Chelation of endogenous zinc depolarizes horizontal cells in situ. Application of Ca-EDTA (1 mM), which chlelates endogenous zinc, depolarized a hybrid striped bass L-type horizontal cell by 3 mV and slightly increased the amplitude of its light responses. Recovery to initial values followed washout. A full-field red (650 nm) light (10^{-1} intensity; 500-ms duration) was used to stimulate the retina. In addition, before and during drug application, a series of full-field 650-nm (indicated by r and r′) and 500 nm (indicated by g and g′) light stimuli of increasing intensity (r, r′: −4 log to −1 log I_o; g, g′: −4 log to log I_o) were flashed onto the retina to assess whether Ca-EDTA altered the amplitude and/or threshold of the cell's light responses. The initial and final dark resting potential of the cell illustrated here was −41 mV.

again, without a change in the Hill coefficient (2.6 ± 0.7 vs. 2.9 ± 0.1).

Zinc chelation depolarizes horizontal cell membrane potentials in the intact retina

Whereas the results described above demonstrate an effect of exogenously applied zinc on retinal neurons, we also wished to ascertain if endogenous zinc in the intact retina acted to modulate glutamatergic transmission to horizontal cells. Ca-EDTA (1 mM) was applied to hybrid bass retinas to chelate endogenous zinc, while intracellular recordings were made from horizontal cells. The dark resting potential of horizontal cells, which is primarily determined by the response of horizontal cells to glutamate released by photoreceptors in the dark (Dowling 1987), was increased by an average of 3.19 ± 0.32 mV ($n = 16$) in the presence of Ca-EDTA (Fig. 5). This is consistent with removal of suppression by endogenous zinc by the chelating action of Ca-EDTA to increase the glutamate responses of horizontal cells. The Ca-EDTA-evoked membrane depolarization was accompanied by a slight increase in the size of the light responses elicited by the two wavelengths tested (i.e., 650 nm: 9.99 ± 2.44%, $n = 16$; 500 nm: 6.91 ± 2.61%, $n = 10$). No effect of Ca-EDTA was observed on the light response threshold.

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.

This differs from findings at other retinal and brain synapses in which zinc reduced glutamate responses when applied at concentrations >300 μM, but actually potentiated responses at lower concentrations (Bresink et al. 1996; Rassendren et al. 1990; Shen and Yang 1999). Whereas our results are unique among native preparations, they are consistent with experiments using heterologous expression of AMPA receptor subunits in which homomeric GluR1 receptors and heteromeric GluR1/GluR2 or GluR2/GluR3 receptors were monotonically inhibited by zinc, while GluR3 homomeric receptors were potentiated at low micromolar zinc concentrations (Dreixler and Leonard 1994, 1997). The modulation of NMDA receptors by zinc also depends on receptor subunit composition; thus there may be mechanistic parallels in AMPA receptor modulation. Low zinc concentrations potentiated homomeric NMDA receptors formed by all type "a" N-methyl-D-aspartic acid receptor subunit 1 (NMDAR1) splice variants while high zinc concentrations inhibited these receptors (Hollmann et al. 1993). In addition, other homomeric or heteromeric NMDA receptors were only inhibited by zinc (Chen et al. 1997; Hollmann et al. 1993).

Bass horizontal cells do not exhibit NMDA or kainate receptor responses, and the precise subunit composition of their AMPA receptors is unknown. The monotonic zinc inhibition, outwardly rectifying current-voltage relation, and cloning of GluR1 homologue from bass retina all suggest the presence of GluR2 and GluR1 heteromers at a minimum (D. G. McMahon and L. Ponomareva, unpublished data). Interestingly, differences in subunit composition may explain the divergent results obtained in carp and bass horizontal cells regarding zinc modulation of glutamate currents. Carp horizontal cells have been shown to express homomeric receptors of GluR3 subunits, which are highly Ca^{2+} permeable (Okada et al. 1999; Schultz et al. 2001). In these neurons, zinc has dual effects with potentiation at low micromolar concentrations and inhibition at high concentrations (Shen and Yang 1999).

Mechanisms of zinc action

Because zinc 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 a zinc-binding site for inhibition seems most likely to be found on the external aspect of receptor, similar to sites known to modulate NMDA and GABA receptors (Choi and

Lipton 1999; Qian et al. 1997; Wang et al. 1995). There is an inhibitory Mg^{2+}-binding site within the pore of NMDA receptor channels (Mayer et al. 1988), and recent data from perch horizontal cells indicate that elevated Mg^{2+} levels can also inhibit the response of non-NMDA receptors in those neurons (Schmidt 1999). However, it is apparent that zinc's action is not through binding to a Mg^{2+}-binding site because zinc inhibition also occurred in the presence of significant Mg^{2+} concentration (1 mM).

Zinc inhibition of AMPA receptor currents persisted in the presence of receptor desensitization blockade, and the rate of receptor desensitization remained unchanged in the presence of zinc 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 ($IC_{50} = 168 \ \mu$M) 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 (Chappell 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

AKAGI T, KANEDA M, ISHII K, AND HASHIKAWA T. Differential subcellular localization of zinc in the rat retina. *J Histochem Cytochem* 49: 87–96, 2001.

ASSAF SY AND CHUNG SH. Release of endogenous zinc from brain tissue during activity. *Nature* 308: 734–736, 1984.

BLANCO R AND DE LA VILLA P. Ionotropic glutamate receptors in isolated horizontal cells of the rabbit retina. *Eur J Neurosci* 11: 867–873, 1999.

BRESINK I, EBERT B, PARSONS CG, AND MUTSCHLER E. Zinc changes AMPA receptor properties: results of binding studies and patch clamp recordings. *Neurophamacology* 35: 503–509, 1996.

CHAPPELL RL AND REDENTI S. Endogenous zinc as a neuromodulator in vertebrate retina: evidence from the retinal slice. *Biol Bull* 201: 265–267, 2001.

CHEN N, MOSHAVER A, AND RAYMOND LA. Differential sensitivity of recombinant *N*-methyl-D-aspartate receptor subtypes to zinc inhibition. *Mol Pharmacol* 51: 1015–1023, 1997.

CHOI YB AND LIPTON SA. Identification and mechanism of action of two histidine residues underlying high-affinity Zn^{2+} inhibition of the NMDA receptor. *Neuron* 23: 171–180, 1999.

CUI C AND MAYER ML. Heteromeric kainate receptors formed by the coassembly of GluR5, GluR6 and GluR7. *J Neurosci* 19: 8281–8291, 1999.

DONEVAN SD AND ROGAWSKI MA. GYKI 52466, a 2,3-benzodiazepine, is a highly selective, noncompetitive antagonist of AMPA/kainate receptor responses. *Neuron* 10: 51–59, 1993.

DONG CJ AND WERBLIN FS. Zinc downmodulates the GABAc receptor current in cone horizontal cells acutely isolated from the catfish retina. *J Neurophysiol* 73: 916–919, 1995.

DOWLING JE. *The Retina: An Approachable Part of the Brain.* Cambridge: Belknapp Press of Harvard University Press, 1987.

DOWLING JE, PAK MW, AND LASATER ER. White perch horizontal cells in culture: methods, morphology and process outgrowth. *Brain Res* 360: 331–338, 1985.

DREIXLER JC AND LEONARD JP. Subunit-specific enhancement of glutamate receptor responses by zinc. *Mol Brain Res* 22: 144–150, 1994.

DREIXLER JC AND LEONARD JP. Effects of external calcium on zinc modulation of AMPA receptors. *Brain Res* 752: 170–174, 1997.

ELIASOF S AND JAHR CE. Rapid AMPA receptor desensitization in catfish cone horizontal cells. *Vis Neurosci* 14: 13–18, 1997.

HOLLMANN M, BOULTER JM, MARON C, BEASLEY L, SULLIVAN J, PECHT G, AND HEINEMANN S. Zinc potentiates agonist-induced currents at certain splice variants of the NMDA receptor. *Neuron* 10: 943–954, 1993.

ISHIDA AT, NEYTON J. Quisqualate and L-glutamate inhibit retinal horizontal-cell responses to kainate. *Proc Natl Acad Sci USA* 82: 1837–1841, 1985.

KNAPP AG AND DOWLING JE. Dopamine enhances excitatory amino acid-gated conductances in cultured retinal horizontal cells. *Nature* 325: 437–439, 1987.

KRUSE M AND SCHMIDT KF. Studies on the dopamine-dependent modulation of amino acid-gated currents in cone horizontal cells of the perch (*perca fluviatilis*). *Vision Res* 33: 2031–2042, 1993.

LASATER EM. Properties of non-NMDA excitatory amino acid-activated channels in isolated retinal horizontal cells. *J Neurosci* 10: 1654–1663, 1990.

LI Y, HOUGH CJ, SUH SW, SARVEY JM, AND FREDERICKSON CJ. Rapid translocation of Zn^{2+} from presynaptic terminals into postsynaptic hippocampal neurons after physiological stimulation. *J Neurophysiol* 86: 2597–2604, 2001.

LU T, SHEN Y, AND YANG XL. Desensitization of AMPA receptors on horizontal cells isolated from crucian carp retina. *Neurosci Res* 31: 123–135, 1998.

MAYER ML, WESTBROOK GL, AND VYKLICKY L JR. Sites of antagonist action on *N*-methyl-D-aspartic acid receptors studied using fluctuation analysis and a rapid perfusion technique. *J Neurophysiol* 60: 645–663, 1988.

MCMAHON DG AND SCHMIT KF. Horizontal cell glutamate receptor modulation by NO: mechanisms and functional implications for the first visual synapse. *Vis Neurosci* 16: 425–433, 1999.

MCMAHON DG, ZHANG DQ, PONOMAREVA L, AND WAGNER T. Synaptic mechanisms of network adaptation in horizontal cells. *Prog Brain Res* 131: 419–436, 2001.

OKADA T, SCHULTZ K, GEURTZ W, HATT H, AND WEILER R. AMPA-preferring receptors with high Ca^{2+} permeability mediate dendritic plasticity of retina; horizontal cells. *Eur J Neurosci* 11: 1085–1095, 1999.

PARTIN KM, PATNEAU DK, WINTERS CA, MAYER BL, AND BUONANNO A. Selective modulation of desensitization at AMPA versus kainate receptors by cyclothiazide and concanavalin A. *Neuron* 11: 1069–1082, 1993.

QIAN H, LI L, CHAPPELL RL, AND RIPPS H. GABA receptors of bipolar cells from the skate retina: actions of zinc on GABA-mediated membrane currents. *J Neurophysiol* 78: 2402–2412, 1997.

RASSENDREN FA, LORY P, PIN JP, AND NARGEOT J. Zinc has opposite effects on NMDA and non-NMDA receptors expressed in *Xenopus* oocytes. *Neuron* 4: 733–740, 1990.

SCHMIDT KF. Divalent cations modulate glutamate receptors in retinal horizontal cells of the perch (*perca fluviatilis*). *Neurosci Lett* 262: 109–112, 1999.

SCHMIDT KF, KRUSE M, AND HATT H. Dopamine alters glutamate receptor desensitization in retinal horizontal cells of the perch (*perca fluviatilis*). *Proc Natl Acad Sci USA* 91: 8288–8291, 1994.

SCHULTZ K, JANSSEN-BIENHOLD U, AND WEILER R. Selective synaptic distribution of AMPA and kainate receptor subunits in the outer plexiform layer of the carp retina. *J Comp Neurol* 435: 433–449, 2001.

SHEN Y AND YANG XL. Zinc modulation of AMPA receptors may be relevant to splice variants in carp retina. *Neurosci Lett* 259: 177–180, 1999.

SPIRIDON M, KAMM D, BILLUPE B, MOBBS P, AND ATTWELL D. Modulation by zinc of the glutamate transporters in glial cells and cones isolated from the tiger salamander retina. *J Physiol (Lond)* 506: 363–376, 1998.

UGARTE M AND OSBORNE NN. The localization of endogenous zinc and the in vitro effect of exogenous zinc on the GABA immunoreactivity and formation of reactive oxygen species in the retina. *Gen Pharmacol* 30: 297–303, 1998.

UGARTE M AND OSBORNE NN. The localization of free zinc varies in rat photoreceptors during light and dark adaptation. *Exp Eye Res* 69: 459–461, 1999.

WANG TL, HACKAM A, GUGGINO WB, AND CUTTING GR. A single histidine residue is essential for zinc inhibition of GABAρ1 receptors. *J Neurosci* 15: 7684–7691, 1995.

WESTBROOK GL AND MAYER ML. Micromolar concentration of Zn^{2+} antagonize NMDA and GABA receptors of hippocampal neurons. *Nature* 328: 640–643, 1987.

WESTERGAARD N, BANKE T, WAHL P, SONNEWALD U, AND SCHOUSBOE A. Citrate modulates the regulation by Zn^{2+} of *N*-methyl-D-aspartate receptor-mediated channel current and neurotransmitter release. *Proc Natl Acad Sci USA* 92: 3367–3370, 1995.

WU SM, QIAO XX, NOEBELS JL, AND YANG XL. Localization and modulatory actions of zinc in vertebrate retina. *Vision Res* 33: 2611–2626, 1993.

YANG JH, MAPLE B, GAO F, MAGUIRE G, AND WU SM. Postsynaptic responses of horizontal cells in the tiger salamander retina are mediated by AMPA-preferring receptors. *Brain Res* 797: 125–134, 1998.

ZHOU LM, GU ZQ, COSTA AM, YAMADA KA, MANSSON PE, GIORDANO T, SKOLNICK P, AND JONES KA. (2S,4R)-4-methylglutamic acid (SYM): a selective, high-affinity ligand for kainate receptors. *J Pharmacol Exp Ther* 280: 422–427, 1997.