

Modulation of glycine receptors in retinal ganglion cells by zinc

(neurotransmitter/synaptic transmission)

YI HAN* AND SAMUEL M. WU

Cullen Eye Institute, Baylor College of Medicine, Houston, TX 77030

Edited by John E. Dowling, Harvard University, Cambridge, MA, and approved January 13, 1999 (received for review December 3, 1998)

ABSTRACT Effects of zinc, an endogenous neuromodulator in the central nervous system, on glycine receptors (GlyRs) in retinal ganglion cells were investigated by using the whole-cell voltage-clamp technique. Zn^{2+} at low concentration ($<2 \mu M$) potentiated the glycine-induced chloride current and at higher concentration ($>10 \mu M$) suppressed it. This biphasic regulatory action of zinc acted selectively on the fast component of the glycine-induced current mediated by the strychnine-sensitive GlyRs, but not on the slow component mediated by the 5,7-dichlorokynurenic acid-sensitive GlyRs. Dose-response studies showed that $1 \mu M Zn^{2+}$ increased the maximum glycine response (I_{∞}) and shifted the EC_{50} to the left, suggesting that Zn^{2+} at low concentrations acts as an allosteric activator of the strychnine-sensitive GlyRs. Zn^{2+} at a concentration of $100 \mu M$ did not alter I_{∞} and shifted the EC_{50} to the right, indicating that Zn^{2+} at high concentrations acts as a competitive inhibitor of the GlyRs. Physiological functions of zinc modulation of GlyRs in retinal ganglion cells are discussed.

The divalent cation Zn^{2+} is an essential element in animal cells. It is not only a cofactor and structural component of more than 200 enzymes but also is an endogenous neuromodulator (1, 2). In the central nervous system, Zn^{2+} is found endogenously in the nerve terminals (3), and it is released on nerve stimulation (4, 5). Evidence in recent years suggests that Zn^{2+} released from nerve terminals can modulate postsynaptic receptors and voltage-gated and ligand-gated channels (1, 2). In this report, we investigated the modulatory actions of zinc on glycine receptors in retinal ganglion cells.

Glycine and γ -aminobutyric acid (GABA) are two major inhibitory neurotransmitters in the central nervous system. Although glycine receptors (GlyR) and GABA receptors (GABAR) are highly homologous, we know much less about GlyR than GABAR. It has been shown that Zn^{2+} regulates GABAR, and the modulatory action depends greatly on the GABAR subunit composition (6, 7). Early studies suggested that mechanisms of zinc modulation on glycine receptors in different tissues follow different patterns. For example, zinc modulations of glycine responses in rat cerebellar granular cells (8) and rat septal cholinergic neurons (9) are monophasic, and zinc regulation of glycine responses in rat embryonic spinal cord neurons is biphasic (10, 11). In rat olfactory bulb neurons, zinc ($100 \mu M$) selectively potentiates the nondesensitizing current but not the steady state of desensitized currents evoked by high concentrations of glycine (12). Furthermore, zinc ($100 nM$ – $10 \mu M$) increases the responses of rat GlyR $\alpha 1$ *in vitro* expressed in *Xenopus* oocytes (13) but biphasically regulates the responses of human GlyR $\alpha 1$ and GlyR $\alpha 2$ expressed in HEK 293 and *Xenopus* oocytes (10, 11).

In a previous study, two components of glycine responses in retinal ganglion cells were identified based on their pharma-

cological and kinetic properties (14). One had a fast onset and rapid desensitization, and the other had a slower onset, much more sustained response. The former was sensitive to low concentrations of strychnine, which is the conventional glycine antagonist; the latter was selectively blocked by $500 \mu M$ 5,7-dichlorokynurenic acid (DCKA) [a blocker of the glycine recognition site at the *N*-methyl-D-aspartate receptor at much lower concentrations (15)]. In this study, we examine whether these two components of glycine responses are differentially regulated by zinc. Endogenous zinc associated with synaptic vesicles has been localized with the Neo-Timm staining method in several types of neurons in the retina, including some in inner retina (16–18), and glycine receptors are known to serve major inhibitory functions in retinal ganglion cells (19, 20). Therefore, our study may provide useful information on how Zn^{2+} shapes ganglion cell responses and how it regulates information processing in the vertebrate retina.

MATERIALS AND METHODS

Cell Preparation. The isolated retinal cell preparation has been described in earlier publications (14, 21). Briefly, the tiger salamander, *Ambystoma tigrinum*, (Kons Scientific, Germantown, WI) was decapitated and pithed, and the eyes were removed. The retina was isolated and incubated for about 30–60 min at room temperature ($22^{\circ}C$) in $400 \mu l$ of enzyme solution containing 20 units/ml lyophilized papain (Worthington, Freehold, NJ), 5 mM L-cysteine, and 400 units/ml DNase I (Worthington, Freehold, NJ) in amphibian Ringer's solution. At the end of the incubation, the retina was rinsed five times with amphibian Ringer's solution and gently shaken until the tissue dissociated. The cells were placed on a lectin-coated coverslip in a culture dish containing Ringer's solution and stored in a $17^{\circ}C$ incubator. Acutely dissociated cells were used in all experiments. Ganglion cells were identified based on a combination of morphological and physiological characteristics, particularly large soma size and magnitude of the inward sodium current (14). Recordings from the retinal slice preparation indicated that presence of tetrodotoxin-sensitive sodium currents and lack of inwardly rectifying currents could distinguish amacrine and ganglion cells from other neurons (N. Tian and M. Slaughter, personal communication). Generally, sodium currents in ganglion cells exceeded those in amacrine cells. These criteria did not positively exclude amacrine cells, but based on the number of cells studied and the consistency of the results, it is reasonable to conclude they are representative of ganglion cell responses. Some dissociated ganglion cells have portions of the axon and dendrites attached, but under our experimental conditions, it was difficult to distinguish between the somatic and dendritic GlyR.

This paper was submitted directly (Track II) to the *Proceedings* office. Abbreviations: GlyR, glycine receptor; GABA, γ -aminobutyric acid; GABAR, GABA receptor; DCKA, 5,7-dichlorokynurenic acid.

*To whom reprint requests should be addressed at: 6565 Fannin, NC 205, Department of Ophthalmology, Baylor College of Medicine, Houston, TX 77030. e-mail: YHAN@BCM.TMC.EDU.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

PNAS is available online at www.pnas.org.

Electrophysiological Recordings. The isolated retinal neurons were bath-perfused with amphibian Ringer's solution consisting of 111 mM NaCl, 3 mM KCl, 2 mM CaCl₂, and 1 mM MgCl₂, buffered with 5 mM Hepes and NaOH, at pH 7.8. The internal pipette solution contained 110 mM potassium gluconate, 5 mM NaCl, 1 mM MgCl₂, and 5 mM EGTA, buffered with 5 mM Hepes and KOH, at pH 7.4. With this internal solution, electrodes had resistance of 5–7 MΩ, and the series resistances were typically 10–20 MΩ (uncompensated). ZnCl₂ was used for Zn²⁺ ions. A rapid perfusion system (BPS-8, ALA Scientific Instruments, Long Island, NY) was used to apply glycine or co-apply glycine and DCKA (Research Biochemicals, Natick, MA). Zn²⁺ and strychnine were added to the bath solution because of their slow actions. Data were recorded with an Axopatch-200A amplifier in combination with a Pentium II computer and CLAMPX7 software (Axon Instruments, Foster City, CA). Offline analysis and data illustration was done by using ORIGIN 5.0 software (Microcal Software, Northampton, MA).

RESULTS

Modulation of Glycine Responses by Zn²⁺. In retinal ganglion cells, glycine induced an outward chloride current at holding potential near 0 mV (Fig. 1; ref. 14). Fig. 1A shows that the peak amplitude of the ganglion cell response to 100 μM glycine was increased from 750 pA (trace *a*) to 1,300 pA (trace *b*) by 1 μM Zn²⁺. On the other hand, Fig. 1B shows that 100 μM Zn²⁺ decreased the peak glycine responses from 600 pA (trace *a*) to 400 pA (trace *b*). This biphasic action on the peak amplitude by Zn²⁺ was observed in all ganglion cells tested. On average, 1 μM Zn²⁺ caused a 1.6-fold (1.6 ± 0.1 , $n = 10$) increase in the peak glycine response and 100 μM Zn²⁺ decreased the peak glycine response to 63% (0.63 ± 0.02 , $n = 10$) of the control level. Zn²⁺ at either concentration exerted no noticeable effect on late phases of glycine-induced currents. Actions of Zn²⁺ at both concentrations were reversible on washout and application of Zn²⁺ alone never induced any current.

The glycine responses in retinal ganglion cells have been pharmacologically and kinetically separated into two different components, suggesting that these cells contain two subtypes of glycine receptors (14). Other evidence has suggested that these two glycine receptor subtypes may have different mo-

lecular subunit compositions (Y.H. and M. Slaughter, unpublished results). It has been known that Zn²⁺ regulates GABA_A receptors, close relatives of the GlyR in the ligand-gated ion channel family, in a way that critically depends on the subunit composition (6, 7). Thus, it is possible that Zn²⁺ differentially modulates different GlyR subtypes. To further investigate this possibility, the glycine antagonists strychnine and DCKA were used to isolate the fast and slow current components. The fast component was mainly mediated by strychnine-sensitive receptors and the slow component by the DCKA-sensitive receptors (14). In the presence of 1 μM strychnine, which selectively blocks the fast glycine responses (14), the remaining slow component was insensitive to both 1 μM Zn²⁺ (Fig. 2A *a* and *b*) and 100 μM Zn²⁺ (Fig. 2A *c* and *d*). On the other hand, when 500 μM DCKA blocked the slow component, 1 μM Zn²⁺ potentiated the fast component (Fig. 2B *a* and *b*), whereas 100 μM Zn²⁺ suppressed it (Fig. 2B *c* and *d*). Fig. 3 summarizes the results shown in Fig. 1 and 2. The modulatory actions of Zn²⁺ disappeared completely in the presence of strychnine but were unaffected by DCKA. These results suggest that zinc selectively regulates the strychnine-sensitive GlyR that mediate the fast component of the glycine response. The DCKA-sensitive receptors mediating the slow component are resistant to zinc modulation.

We next examined the Zn²⁺ concentration dependence of its modulation on glycine-induced currents. Effects of Zn²⁺ were measured by normalizing the glycine-induced current in the presence of Zn²⁺ against the current in control Ringer's solution ($I_{Zn^{2+}}/I_{ctr}$). Because the early peaks of glycine responses were mainly strychnine-sensitive and the late, steady phases were predominately DCKA-sensitive (14), the peak responses were used to represent the fast glycine responses and the plateaus were used to indicate the slow ones. As shown in Fig. 4, in all cells tested, the biphasic modulation by Zn²⁺ acted on the fast component but not on the slow component of the glycine response. Zn²⁺ at concentrations <100 nM did not affect the glycine response, and at 0.5–1 μM potentiated glycine responses. Higher concentrations of Zn²⁺ reduced the potentiation, followed by inhibition of the glycine response at concentrations greater than 2 μM. Consequently, low concentrations of Zn²⁺ made glycine-induced currents more transient, and high concentrations of Zn²⁺ made them more sustained (see Fig. 1, for example). Such regulation of the

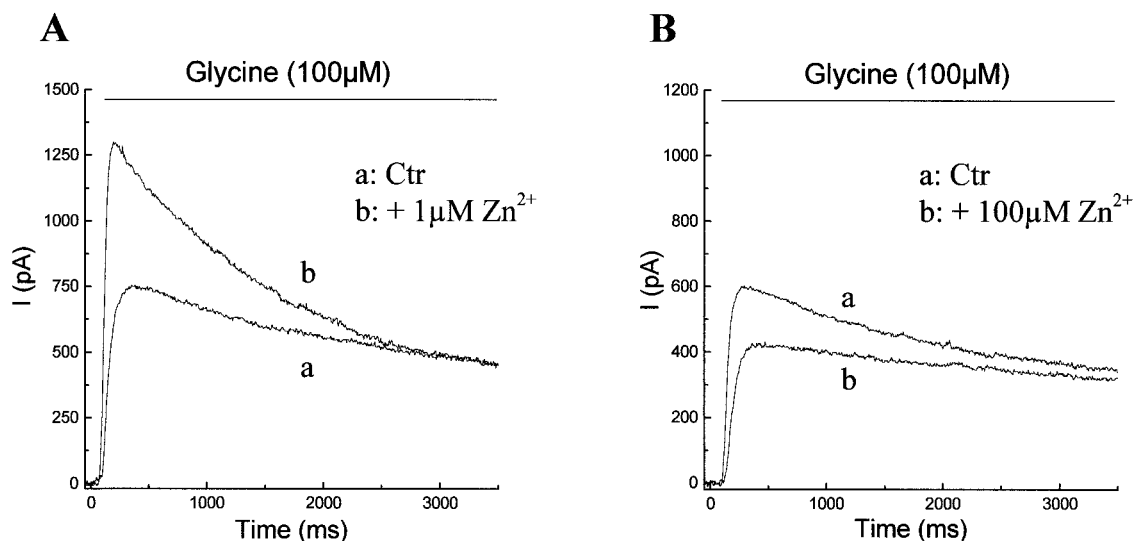


FIG. 1. Effects of 1 μM (*A*) and 100 μM (*B*) Zn²⁺ on the current responses to 100 μM glycine in retinal ganglion cells. In each case, current responses were recorded under voltage-clamp conditions at a holding potential of 0 mV. Glycine-induced currents in control Ringer's solution were presented as trace *a*, and currents in Ringer's solution containing 1 μM (*A*) or 100 μM (*B*) were shown as trace *b*.

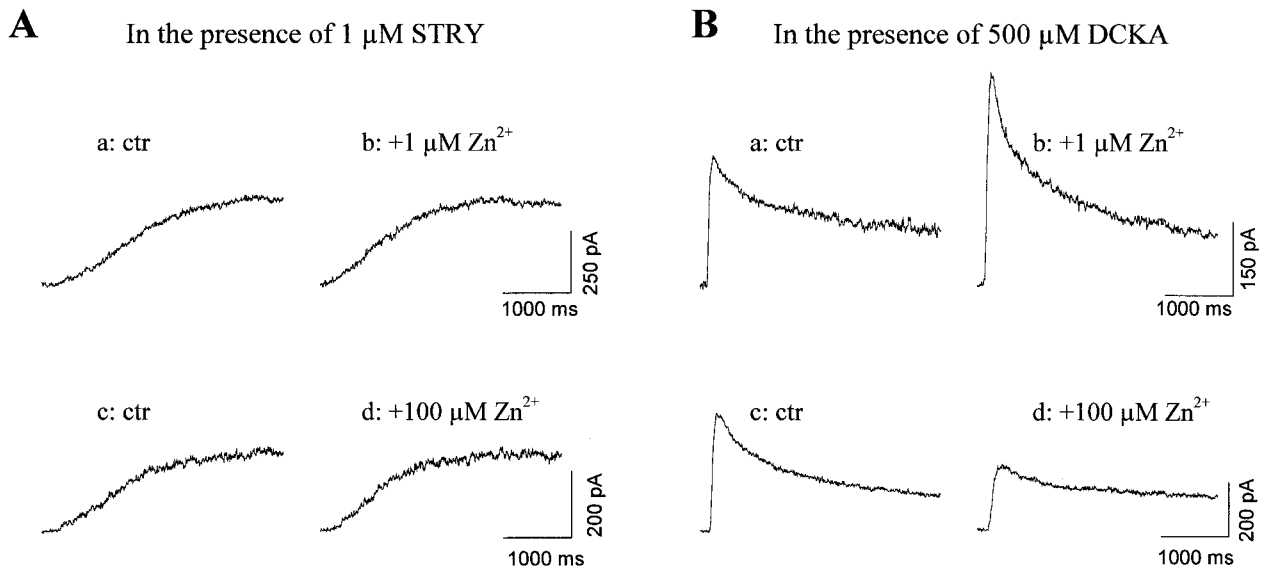


FIG. 2. Differential modulation of glycine receptor subtypes by Zn^{2+} . (A) With 1 μM strychnine, the fast component of the glycine response was blocked (traces *a* and *c*), the slow component in 1 μM Zn^{2+} were shown as trace *b*, and in 100 μM Zn^{2+} were shown as trace *d*. (B) In the presence of 500 μM DCKA, the slow component was blocked (trace *a* and *c*), and trace *b* and *d* illustrate the fast component in 1 μM Zn^{2+} and 100 μM Zn^{2+} , respectively. In each case, trace *a* and *b* were from the same cell, as were trace *c* and *d*. All current responses were recorded under voltage-clamp conditions at a holding potential of 0 mV.

glycine response waveform was observed at all concentrations of zinc tested in this study.

Mechanism of Zn^{2+} Regulation on Fast Component of Glycine Responses. To unravel the mechanism of Zn^{2+} interaction with the GlyR subtype mediating fast glycine responses, we analyzed Zn^{2+} modulation on the glycine dose-response relationship. In semilogarithmic dose-response plots, the current responses increased with glycine concentrations following a sigmoidal equation (Fig. 5A and B, and Eq. 1 in Appendix). The shapes of these dose-response curves in various concentrations of Zn^{2+} were similar, indicating that Zn^{2+} did not significantly change the Hill coefficient ($n_H \approx 2$). Zn^{2+} (1 μM) facilitated glycine binding to GlyR and shifted EC_{50} to the left ($EC_{50, Ctr} = 43 \mu M$, $EC_{50, Zn^{2+}} = 35 \mu M$), suggesting that Zn^{2+}

at low concentrations acts as an allosteric activator. It also increased the maximum glycine response ($I_{Ctr, \infty} = 360$ pA, $I_{Zn^{2+}, \infty} = 580$ pA) (Fig. 5A). In contrast, 100 μM Zn^{2+} did not affect the maximum glycine responses, but it acted as a competitive inhibitor for glycine receptors, lowered the apparent glycine affinity, and shifted EC_{50} to the right (Fig. 5B; $EC_{50, Ctr} = 40 \mu M$, $EC_{50, Zn^{2+}} = 74 \mu M$).

The relationship between Zn^{2+} modulation on glycine responses and glycine concentration ($[Gly]$) was studied by plotting $I_{Zn^{2+}}/I_{Ctr}$ as a function of $[Gly]$ in Fig. 5C. At low concentrations of glycine, 1 μM Zn^{2+} potentiated the glycine response >2 -fold ($I_{Zn^{2+}}/I_{Ctr} = 2.17 \pm 0.03$, $n = 3$, at $[Gly] = 25 \mu M$). As $[Gly]$ increased, the current potentiation induced by 1 μM Zn^{2+} decreased progressively, and $I_{Zn^{2+}}/I_{Ctr}$

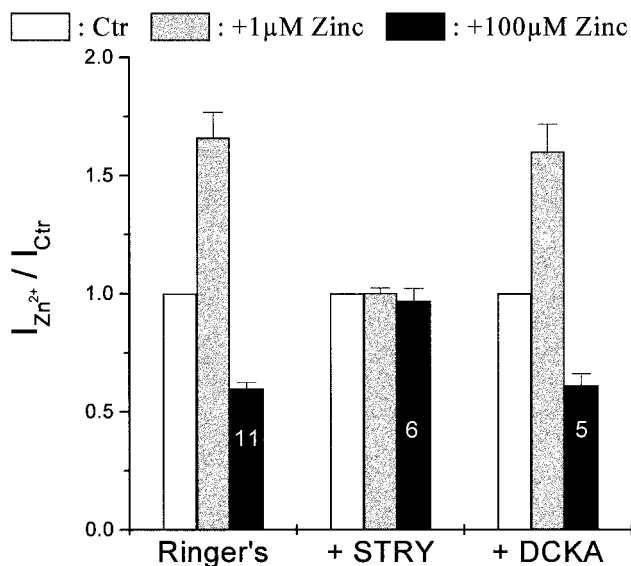


FIG. 3. Histograms of the Zn^{2+} effects under different conditions, $I_{Zn^{2+}}/I_{Ctr}$ was used to quantify Zn^{2+} modulation on glycine-induced currents. The number inside the columns indicates the number of cells tested under those conditions. The bars above the columns represented the SE.

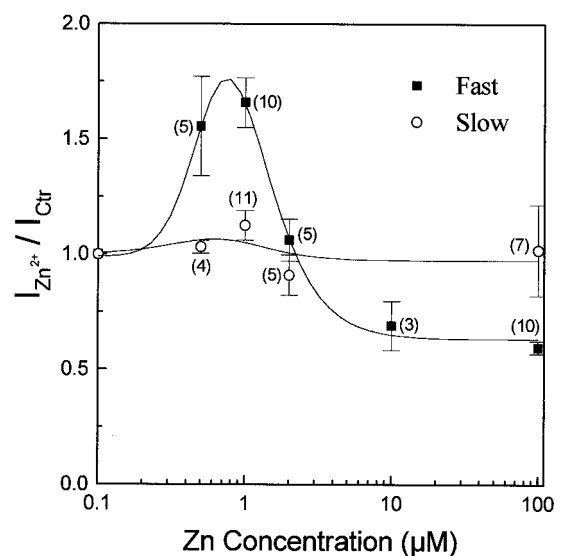


FIG. 4. Dose-related Zn^{2+} modulation on glycine responses. The fast (peak) component of glycine (■) and the slow (sustained) component (○) responses were plotted against zinc concentration. Each data point represented the mean \pm SE from multiple cells. The number near each data point indicates the number of cells tested at that concentration.

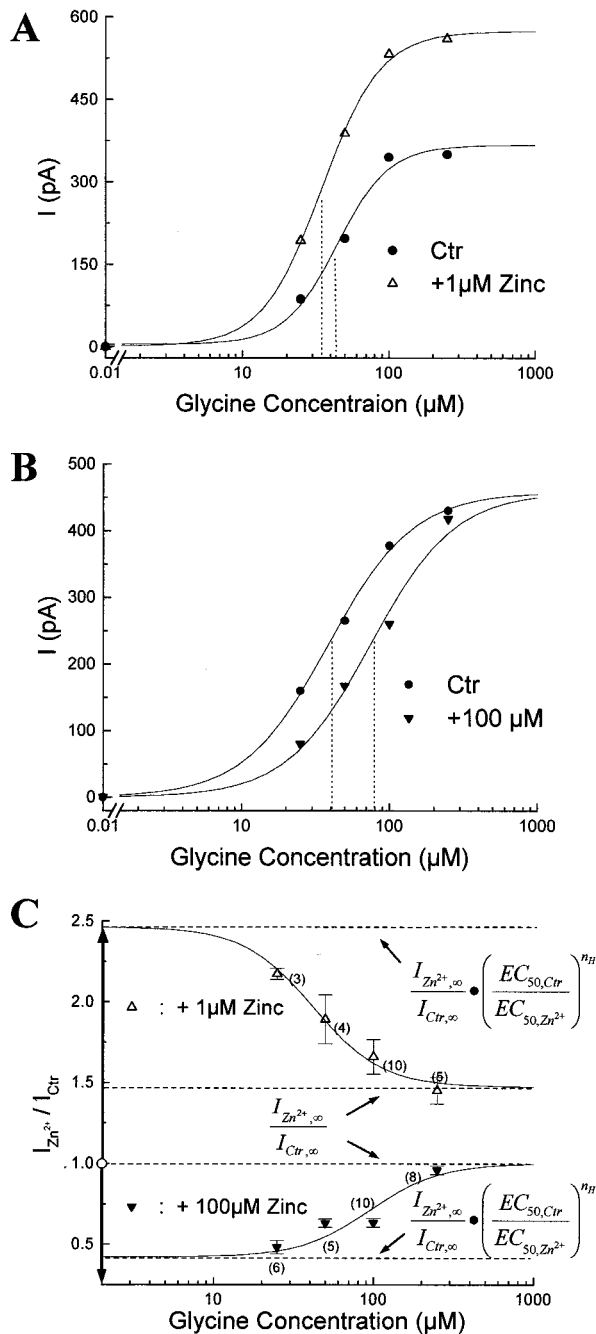


FIG. 5. Dose-response curves of glycine-induced currents (I vs. $[\text{Gly}]$) in the absence and presence of $1 \mu\text{M}$ Zn^{2+} (A) and $100 \mu\text{M}$ Zn^{2+} (B). Glycine dose-response curves were fitted by following equation (where I_{∞} is the maximum glycine-induced current, I_0 is the initial current when $[\text{Gly}]$ is near zero, n_H is the Hill coefficient):

$$I = I_0 + \frac{I_{\infty} - I_0}{1 + (EC_{50}/[\text{Gly}])^{n_H}}$$

The relative current $I_{\text{relative}} = I_{\text{Zn}^{2+}}/I_{\text{ctr}}$ in $1 \mu\text{M}$ Zn^{2+} (Δ) and in $100 \mu\text{M}$ Zn^{2+} (\blacktriangledown) was plotted as a function of $[\text{Gly}]$ in C. The horizontal dashed lines indicate the initial relative current ($I_{\text{relative},0}$) and maximum relative current ($I_{\text{relative},\infty}$) in Eq. 4 in the Appendix. The number near each data point indicates the number of cells tested at that concentration.

I_{ctr} reached a steady state level of about 1.45 ± 0.08 ($n = 5$) at $[\text{Gly}] = 250 \mu\text{M}$. On the other hand, $100 \mu\text{M}$ Zn^{2+} suppressed the glycine response by more than one-half when low concentrations of glycine were employed ($I_{\text{Zn}^{2+}}/$

$I_{\text{ctr}} = 0.48 \pm 0.02$, $n = 6$, at $[\text{Gly}] = 250 \mu\text{M}$). The current suppression induced by $100 \mu\text{M}$ Zn^{2+} also decreased as $[\text{Gly}]$ increased. Eventually, $100 \mu\text{M}$ Zn^{2+} lost the inhibitory effect on currents induced by high $[\text{Gly}]$ ($I_{\text{Zn}^{2+}}/I_{\text{ctr}} = 0.96 \pm 0.02$, $n = 8$ at $[\text{Gly}] = 250 \mu\text{M}$). Because Zn^{2+} changed the apparent glycine affinity but not the Hill coefficient, the $I_{\text{Zn}^{2+}}/I_{\text{ctr}}$ -vs.- $[\text{Gly}]$ plot also follows a sigmoidal relationship described by Eq. 3 in the appendix. By fitting Eq. 3 with data points in Fig. 3C, we found that the apparent glycine affinity increased by $\approx 30\%$ with $1 \mu\text{M}$ Zn^{2+} , and decreased by 35% with $100 \mu\text{M}$ Zn^{2+} (See Appendix, based on $n_H = 2$). The EC_{50} for glycine is $\approx 40 \mu\text{M}$ with $1 \mu\text{M}$ Zn^{2+} and approximately $100 \mu\text{M}$ with $100 \mu\text{M}$ Zn^{2+} .

DISCUSSION

Zn^{2+} Modulation of GlyRs on Retinal Ganglion Cells. The data presented in this paper constitute demonstration that Zn^{2+} selectively modulates a subtype of GlyR in retinal ganglion cells and that this modulation is biphasic. At low concentrations, Zn^{2+} potentiates the glycine-induced currents and at high concentrations suppresses the currents. Such dose-dependent biphasic modulation of GlyRs by Zn^{2+} have been observed in spinal cord neurons and recombinant GlyRs expressed in HEK 293 cells (10, 11). The concentration ranges of Zn^{2+} for potentiation and suppression of GlyRs from these other studies are very close to those in our study.

The GlyR is a pentamer, and different subtypes of GlyRs may have different subunit compositions (22–24). Our data show that Zn^{2+} selectively modulates the strychnine-sensitive GlyRs but not the DCKA-sensitive GlyRs. This is in agreement with the results obtained from other CNS preparations, which suggest that the binding sites for Zn^{2+} and strychnine are both located in the α -subunit (10, 11). In contrast, the DCKA-sensitive GlyRs may contain different α -subunits or other subunits that block the Zn^{2+} -binding site and thus make them resistant to Zn^{2+} modulation.

For the strychnine-sensitive GlyRs, Zn^{2+} may have two binding sites with different affinities because Zn^{2+} regulates glycine responses biphasically. This idea also is consistent with the results from chimeric constructs of GlyRs (11). At low concentrations, Zn^{2+} binds to the high-affinity site, which facilitates glycine binding as well as interferes with channel gating, causing the increase of whole-cell conductance. As the concentration increases, Zn^{2+} binds to the low-affinity site. This binding may be more stable, thus inhibiting Zn^{2+} binding to the high-affinity site. The low-affinity site may be located somewhere near the glycine-binding pocket, and thus it acts as a competitive inhibitor for the GlyR. In either case, the Hill coefficient of the glycine dose-response curve is not altered by Zn^{2+} , suggesting that the number of glycine molecules required for channel gating is unaffected by Zn^{2+} modulation.

Physiological Function of Zn^{2+} Modulation in Retinal Ganglion Cells. It has been known for a long time that zinc exists in high amounts in the retina (25). Recent studies using the Neo-Timm method have shown that endogenous zinc associated with synaptic vesicles is located in photoreceptors of tiger salamander, fish, and mammalian retinas (16–18). Moreover, these studies revealed zinc-containing processes in the inner plexiform layer, although their cellular origin remains uncertain (16, 18). These results suggest that retinal ganglion cells may be influenced by zinc released from neuronal processes in the inner retina and by zinc diffused from photoreceptors in the distal retina. In hippocampal neurons, vesicular zinc is coreleased with glutamate on membrane depolarization (4, 5). In darkness, photoreceptors are depolarized and others are hyperpolarized (26, 27). Therefore, darkness increases zinc release from photoreceptors,

and it may increase or decrease zinc release from neuronal processes in the inner retina. In the salamander outer retina, it has been shown that bath application of 5 μM Zn^{2+} substantially suppresses synaptic transmission between photoreceptors and horizontal cells (16). Therefore, extracellular Zn^{2+} concentration in dark-adapted retinas under physiological conditions while synaptic transmission is not suppressed must be substantially lower than 5 μM . This leads us to propose that under physiological conditions, Zn^{2+} released from retinal neurons primarily potentiates glycine responses in ganglion cells by binding to the high-affinity binding site. However, we cannot exclude the possibility that local zinc concentration in some part of the retina is in the high micromolar range. It has been shown that zinc release and uptake vary with neuronal activity (4, 5), and local concentrations of zinc may reach the high micromolar range (300 μM) in the hippocampus (28). If such high zinc concentrations exist near retinal ganglion cells, then Zn^{2+} may suppress glycine responses by binding to the low-affinity binding site.

Our results (Fig. 1) indicate that low concentrations of Zn^{2+} made glycine-induced currents more transient and high concentrations of Zn^{2+} made them more sustained. Because ganglion cells receive glycinergic inputs from transient amacrine cells, which contribute to the antagonistic surround response and motion sensitivity of retinal ganglion cells (20), the dose-dependent biphasic Zn^{2+} modulation of GlyRs may be used to adjust the center-surround antagonistic receptive field and motion detection capability of retinal ganglion cells.

APPENDIX

Glycine dose-response curves are sigmoidal and can be fitted by the following equation:

$$I = I_0 + \frac{I_\infty - I_0}{1 + (EC_{50}/[\text{Gly}])^{n_H}} \quad [1]$$

When $I_0 = 0$, the above equation becomes:

$$\frac{I}{I_\infty} = \frac{[\text{Gly}]^{n_H}}{(EC_{50})^{n_H} + [\text{Gly}]^{n_H}} \quad [2]$$

If Zn^{2+} does not change n , the relative current,

$$I_{\text{relative}} = \frac{I_{\text{Zn}^{2+}}}{I_{\text{Ctr}}} = \frac{I_{\text{Zn}^{2+},\infty}}{I_{\text{Ctr},\infty}} \times \frac{(EC_{50,\text{Ctr}})^{n_H} + [\text{Gly}]^{n_H}}{(EC_{50,\text{Zn}^{2+}})^{n_H} + [\text{Gly}]^{n_H}} \quad [3]$$

Rearranging parameters in Eq. 3, we obtain

$$I_{\text{relative}} = \frac{I_{\text{Zn}^{2+},\infty}}{I_{\text{Ctr},\infty}} \times \left(\frac{EC_{50,\text{Ctr}}}{EC_{50,\text{Zn}^{2+}}} \right)^{n_H} + \frac{\frac{I_{\text{Zn}^{2+},\infty}}{I_{\text{Ctr},\infty}} - \frac{I_{\text{Zn}^{2+},\infty}}{I_{\text{Ctr},\infty}} \times \left(\frac{EC_{50,\text{Ctr}}}{EC_{50,\text{Zn}^{2+}}} \right)^{n_H}}{1 + \left(\frac{EC_{50,\text{Zn}^{2+}}}{[\text{Gly}]} \right)^{n_H}} \quad [4]$$

Comparing Eqs. 1 and 4 shows that the I_{relative} -vs.-glycine

concentration plot is also sigmoidal, with

$$I_{\text{relative},0} = \frac{I_{\text{Zn}^{2+},\infty}}{I_{\text{Ctr},\infty}} \times \left(\frac{EC_{50,\text{Ctr}}}{EC_{50,\text{Zn}^{2+}}} \right),$$

$$I_{\text{relative},\infty} = \frac{I_{\text{Zn}^{2+},\infty}}{I_{\text{Ctr},\infty}}, \text{ and}$$

$$EC_{50,\text{relative}} = EC_{50,\text{Zn}^{2+}}.$$

We thank Dr. Roy Jacoby for reading this manuscript. This work is supported by grants from the National Institutes of Health (EY04446), the Retina Research Foundation (Houston), and The Research to Prevent Blindness, Inc.

- Smart, T. G., Xie, X. & Krishek, B. J. (1994) *Prog. Neurobiol.* **42**, 393–341.
- Harrison, N. L. & Gibbons, S. J. (1994) *Neuropharmacology* **33**, 935–952.
- Slomianka, L. (1992) *Neuroscience* **48**, 325–352.
- Assaf, S. Y. & Chung, S. H. (1984) *Nature (London)* **308**, 734–736.
- Howell, G. A., Welch, M. G. & Frederickson, C. J. (1984) *Nature (London)* **308**, 736–738.
- Draguhn, A., Verdorn, T. A., Ewert, M., Seeburg, P. H. & Sakmann, B. (1990) *Neuron* **5**, 781–788.
- Smart, T. G., Moss, S. J., Xie, X. & Haganir, R. L. (1991) *Br. J. Pharmacol.* **103**, 1837–1839.
- Virginio, C. & Cherubini, E. (1997) *Brain Res. Dev. Brain Res.* **98**, 30–40.
- Kumamoto, E. & Murata, Y. (1996) *J. Neurophysiol.* **76**, 227–241.
- Bloomenthal, A. B., Goldwater, E., Pritchett, D. B. & Harrison, N. L. (1994) *Mol. Pharmacol.* **46**, 1156–1159.
- Laube, B., Kuhse, J., Rundstrom, N., Kirsch, J., Schmieden, V. & Betz, H. (1995) *J. Physiol. (London)* **483**, 613–619.
- Trombley, P. Q. & Shepherd, G. M. (1996) *J. Neurophysiol.* **76**, 2536–2546.
- Akagi, H., Majima, T. & Uchiyama, M. (1994) *Jpn. J. Physiol.* **44** Suppl., S91–S96.
- Han, Y., Zhang, J. & Slaughter, M. M. (1997) *J. Neurosci.* **17**, 3392–3400.
- Kemp, J. A., Foster, A. C., Leeson, P. D., Priestley, T., Tridgett, R., Iversen, L. L. & Woodruff, G. N. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6547–6550.
- Wu, S. M., Qiao, X., Noebels, J. L. & Yang, X. L. (1993) *Vision Res.* **33**, 2611–2616.
- Qian, H., Li, L., Chappell, R. L. & Ripps, H. (1997) *J. Neurophysiol.* **78**, 2402–2412.
- Ugarte, M. & Osborne, N. N. (1998) *Gen. Pharmacol.* **30**, 297–303.
- Miller, R. F., Frumkes, T. E., Slaughter, M. & Dacheux, R. F. (1981) *J. Neurophysiol.* **45**, 764–782.
- Belgium, J. H., Dvorak, D. R. & McReynolds, J. S. (1984) *J. Physiol.* **354**, 273–286.
- Han, Y. & Slaughter, M. M. (1998) *J. Physiol. (London)* **508**, 681–690.
- Betz, H., Langosch, D., Hoch, W., Prior, P., Pribilla, I., Kuhse, J., Schmieden, Malosio, M. L., Matzenbach, B. & Holzinger, F. (1991) *Adv. Exp. Med. Biol.* **287**, 421–429.
- Betz, H. (1992) *Q. Rev. Biophys.* **25**, 381–394.
- Betz, H., Kuhse, J., Fischer, M., Schmieden, V., Laube, B., Kuryatov, A. L., Meyer, G., Bormann, J. & Rundstrom, N. (1994) *J. Physiol. (Paris)* **88**, 243–248.
- Eckert, C. D. (1979) *Fed. Proc.* **392**, 872 (abstr.).
- Werblin, F. S. & Dowling, J. E. (1969) *J. Neurophysiol. (London)* **32**, 339–355.
- Kaneko, A. (1970) *J. Physiol. (London)* **207**, 623–633.
- Frederickson, C. J., Klitenick, M. A., Manton, W. I. & Kirkpatrick, J. B. (1983) *Brain Res.* **273**, 335–339.