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Zinc Modulation of Calcium Activity at the Photoreceptor Terminal: A Calcium Imaging Study

Ivan Anastassov^{a,b,1}, Wen Shen^{b,c}, Harris Ripps^{b,d}, and Richard L. Chappell^{a,b,e,*}

^aDepartment of Biological Sciences, Hunter College and the Graduate Center, CUNY, 695 Park Avenue, New York, NY 10065, USA, ianastassov77@gmail.com

^bMarine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02543, USA

^cDepartment of Biomedical Science, CES College of Medicine, Florida Atlantic University Medical School, Boca Raton, FL 33431, USA, wenshen2008@gmail.com

^dDepartments of Ophthalmology and Visual Sciences, Anatomy and Cell Biology, and Physiology and Biophysics, University of Illinois College of Medicine, Chicago, IL 60612, USA, harrripp@uic.edu

^eCenter for Vision Research, Brown Institute for Brain Science, Box 1953, Providence, RI 02912, USA

Abstract

There is abundant experimental evidence that zinc ions (Zn^{2+}) are present in the synaptic vesicles of vertebrate photoreceptors, and that they are co-released with glutamate. Here we show that increasing the concentration of extracellular zinc (2 μ M to 2 mM) suppresses the entry of calcium into the synaptic terminals of isolated salamander double cones. The resultant dose-dependent curve was fit by an inverse Hill equation having an IC_{50} of 38 μ M, and Hill coefficient of 1.1. Because there is currently no reliable way to measure the concentration of extracellular zinc, it is not known whether the zinc released under normal circumstances is of physiological significance. In an attempt to circumvent this problem we used zinc chelators to reduce the available pool of endogenous zinc. This enabled us to determine how the *absence* of zinc affected calcium entry. We found that when intra- or extra-cellular zinc was chelated by 250 μ M of membrane-permeable TPEN or 500 μ M of membrane-impermeable histidine, there was a significant rise in the depolarization-induced intracellular calcium level within photoreceptor terminals. This increase in internal $[Ca^{2+}]$ will undoubtedly lead to a concomitant increase in glutamate release. In addition, we found that blocking the L-type calcium channels that are expressed on the synaptic terminals of photoreceptors with 50 μ M nifedipine or 100 μ M verapamil abolished the effects of zinc chelation. These findings are a good indication that, when released *in vivo*, the zinc concentration

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*Corresponding author: Marine Biological Laboratory 7 MBL Street Woods Hole, MA 02543 USA rchappell@MBL.edu Tel: +1 508 289 7664 Fax: +1 508 289 7934 .

¹Present address: Department of Biochemistry and Molecular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA

Author contributions IA conducted the experiments, and all authors analyzed the results, contributed to the design of the study, and helped with the preparation of the manuscript. Experiments were conducted at the Marine Biological Laboratory, Woods Hole, MA.

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is sufficient to suppress voltage-gated calcium channels, and reduce the rate of glutamate release from photoreceptor terminals.

Keywords

zinc chelation; synaptic terminals; calcium imaging; photoreceptors; voltage-gated calcium channels

1. Introduction

Zinc is one of the most ubiquitous and important trace elements in biological systems, and it has proven indispensable to the growth and development of all forms of life (Hambidge, 1981; Vallee, 1988). Its importance stems largely from the fact that zinc is an integral and essential component of scores of enzymes and thus participates in a broad range of metabolic functions (Vallee and Auld, 1990), as well as playing a significant role in translation and transcription of the genetic message (Vallee and Falchuk, 1981; O'Halloran, 1993).

Less well known is the physiological significance of Zn^{2+} located in the synaptic terminals of glutamatergic neurons. Experimental study of the role of ionic zinc has been severely impeded by the fact that Zn^{2+} was considered a quantitatively immeasurable trace element, and by a lack of analytical methods for its detection and localization. With the availability of sensitive and reliable methods for histochemical imaging, the presence of "chelatable" or "free" zinc was shown to be within the synaptic vesicles of glutamatergic nerve terminals in the hippocampus (Aniksztejn et al., 1987; Ketterman and Li, 2008), in specific layers of the cerebral cortex and other regions of the CNS (Frederickson and Danscher, 1990; Sensi et al., 2009), and in the synaptic terminals of photoreceptors in the vertebrate retina (Wu et al., 1993; Qian et al., 1997; Ugarte and Osborne, 1998). Moreover, the presence of the vesicle-associated zinc transporter 3 (ZnT-3, Cole et al., 1999) in the synaptic region of photoreceptors (Redenti and Chappell, 2004) suggested that ionic zinc is co-localized with glutamate within the vesicles of the synaptic terminal. This has since been confirmed, and there is now evidence that the co-release of glutamate and ionic zinc is a calcium-dependent process (Frederickson and Bush, 2001; Gee et al., 2002; Redenti et al., 2007).

The inability to determine the concentration of extracellular Zn^{2+} at post-synaptic sites has clouded an evaluation of its physiological significance as an effective participant in neuronal activity. We have attempted to circumvent the uncertainty regarding ion concentration by analyzing the effects induced by *removal* of endogenous zinc from synaptic sites; we were then able to compare results obtained under "normal" conditions with those observed after zinc chelation. Using this approach in conjunction with calcium-imaging microscopy of photoreceptor cells, we could determine changes in intracellular calcium when zinc chelation occurs either inside or outside the cell.

2. Methods

All procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996, and were approved by the Institutional Animal Care and Use Committee of the Marine Biological Laboratory Woods Hole, MA in accordance with its guidelines.

2.1 Cell dissociation and dye loading

Tiger salamanders (*Ambystoma tigrinum*), obtained from Kons Scientific (Germantown, WI) and Charles Sullivan (Nashville, TN), were used in this study. The salamanders were kept at 4–8°C on a 12 hour light-dark cycle. Prior to tissue extraction, animals were anesthetized with 0.1% tricaine methanesulfonate (MS-222, Argent Chemical, Redmond, WA) and decapitated. Eyes were enucleated under ambient light, the cornea and lens were removed, and the retina was isolated. The retinal tissue was enzymatically dissociated by gentle agitation in Ringer solution containing 12 U/ml papain (Worthington Biochemical, Lakewood, NJ) activated with 5 mM L-cysteine (Calbiochem, La Jolla, CA), and adjusted to pH 7.4. It was then triturated through a fire-polished Pasteur pipette to obtain isolated neurons. Cells were plated on 35 mm #1.5 glass bottom dishes (In Vitro Scientific, Sunnyvale, CA), coated with 1 mg/ml lectin from *Canavalia ensiformis* (Sigma Aldrich, St. Louis, MO), and allowed to adhere for 10–20 min. The cells were then immersed for 30 min at 10°C in the membrane permeable calcium indicator dye Fluo-4, AM (Molecular Probes, Inc., Eugene, OR), dissolved in high quality 99.7% anhydrous dimethylsulfoxide (DMSO, Acros Organics, Fairlawn, NJ) which was diluted with Ringer to a 0.5 – 1 μ M working concentration. The Ringer solution contained (in mM): NaCl (111), KCl (3), CaCl₂ (2), MgCl₂ (1), Dextrose (10), HEPES (5), (all from Fisher Scientific, Pittsburgh, PA), pH 7.7. After incubation, during which time Fluo-4 AM was internalized and the acetoxymethyl (AM) esters were cleaved to form the free Fluo-4, the external Fluo-4 AM solution was washed off and replaced with normal Ringer solution for calcium imaging. All other chemicals and solutions (nicardipine hydrochloride, verapamil hydrochloride, L-histidine, N,N,N',N'-Tetrakis (2-pyridylmethyl) ethylenediamine (TPEN)) were purchased from Sigma-Aldrich (St Louis, MO).

2.2 Ca²⁺ imaging

Isolated, dye-loaded photoreceptor cells were imaged using a Zeiss Axiovert 200M microscope with a 40X Plan Neofluor 0.6 NA dry objective (Carl Zeiss Vision GmbH, Munich, Germany). Images were collected continuously with a Zeiss AxioCam MRm CCD camera and displayed with AxioVision 4.8.2 software. A Zeiss HXP 120 mercury halide light source with a built-in shutter was used for dye excitation. Images were collected every second with an exposure time of 150 ms. The double cone somas and their bulging synaptic terminals were selected as areas of interest (AOI) and the change in average fluorescence signal intensity of the selected area was plotted over time. Peak intensities at every drug application were compared graphically and statistically. Drugs were delivered via a pressurized perfusion system (ALA-VM8, ALA Scientific, Farmingdale, NY) with fast pinch- or solenoid valve-controlled solution changes allowing all test solutions to be delivered for a period of 10 sec. The solutions were oxygenated by using an oxygen tank as the pressure source for perfusion. The system allowed accurate replication of the perfusion rate (1 ml/min) between experiments, and the volume of the delivery manifold was ~5 μ l.

2.3 Data analysis

Statistical analysis of calcium recordings was performed using GraphPad Prism software, version 5.04. All trials started with a control application of 30 mM KCl alone and AOI peak intensities for every cell were normalized to the first application of KCl. A control group of cells (n=18) received only KCl treatments at regular intervals, while treatment groups received KCl with drugs at the same intervals following the initial (control) KCl pulse. A 1-way ANOVA test, performed on all treatment groups, showed overall significance values of $p < 0.05$. Subsequently, each peak in the treatment group was compared to its sister peak in the control group using a two-tailed unpaired student *t*-test. Error bars shown in the figures are $\pm$ SEM.

Since photo-bleaching caused a small time-dependent decline in the fluorescence signal (see Fig. 1C), we were able to construct an average decay curve of peak fluorescence intensity with time (not shown) based on repeated applications of 30 mM KCl alone. This enabled us to correct for changes in fluorescent intensity due to bleaching, and any reduction in fluorescent signal could be attributed solely to drug application. Decay coefficients were only used for fitting the zinc dose-response of Fig. 2D.

3. Results

3.1 Depolarization-induced calcium influx in photoreceptor terminals

Photoreceptors, like other neurons, release their transmitter when depolarized. This vesicular release is triggered by calcium entry through voltage-gated calcium channels, and numerous studies have confirmed the presence of L-type calcium channels on the photoreceptor terminals of salamander (Steele et al., 2005) and other vertebrate species (Morgans et al., 2005; Mercer et al., 2011). In this way, synaptic release of glutamatergic vesicles is tightly coupled to an increase of calcium levels in the terminal. To monitor calcium level changes in photoreceptor terminals, we repeatedly applied 30 mM KCl to depolarize photoreceptors, and recorded the temporal changes in intracellular calcium by means of the fluorescent intensity changes of Fluo-4. Fig. 1A shows a number of time points on the rising and falling phases of the depolarizing response to KCl. The series of calcium images shown in Fig. 1B illustrate the fluorescent changes corresponding to each numbered time point in Fig. 1A; i.e., from the initial influx of calcium, to the peak, and the subsequent decline to baseline. It is evident that calcium entry takes place initially in the synaptic terminal, and over time diffuses internally throughout the visual cell. The sequence of calcium traces shown in Figs. 1C and D represent similar results obtained in response to repetitive stimulation. Each depolarizing pulse of KCl produced a rise in intracellular calcium, but note that the amplitudes of the calcium peaks were quite similar, indicating that decay due to photo-bleaching was minimal.

3.2 Changes in calcium influx in response to applied zinc

To determine the influence of extracellular zinc on transmitter release, we examined its effect on the entry of calcium in photoreceptor terminals depolarized by the application of KCl. We found that the response to 30 mM KCl (Fig. 2A, peak 2) was greatly attenuated by the co-application of 100 μ M zinc (Fig. 2A, peak 4). After washout, there was a reduced response to KCl (Fig. 2A, peak 6). Co-application of KCl and nifedipine, a dihydropyridine analog that specifically blocks L-type calcium channels, abolished the depolarization-induced intracellular calcium increase. This is a good indication that the entry of calcium in photoreceptor terminals occurs mainly via L-type calcium channels. The corresponding Fluo 4 images of calcium changes in Fig. 2A are shown in Fig. 2B, and the bar graphs in Fig. 2C show the averaged data for multiple trials with each experimental condition. In order to analyze quantitatively the suppressive effect of zinc on the KCl-induced intracellular calcium increase in photoreceptor terminals, we applied a range of zinc concentrations (from 2 μ M to 2 mM) together with 30 mM KCl. Fig. 2D shows the dose-dependent suppression of KCl-induced intracellular calcium increase in double cone terminals by external zinc. The dose-response data were well fit by a modified Hill equation of the form: $I/I_{\max} = I_{\max} - \{[C]^p / ([C]^p + [IC_{50}]^p)\}$ where I is the intensity of the calcium signal, $I_{\max}$ is its maximum value, C is the zinc concentration, p is the Hill coefficient, and IC_{50} is the concentration that reduces the calcium response by 50%. The results indicated that the IC_{50} was produced by 38 μ M zinc with a Hill coefficient of 1.1. These results suggest that micromolar concentrations of zinc can effectively suppress calcium entry, and thus reduce calcium-dependent glutamate release from photoreceptor terminals.

3.3 Effects of zinc chelation

Having demonstrated that exogenous zinc reduces calcium entry into photoreceptors, it was essential to determine whether the *removal of endogenous zinc* (Zn^{2+}) would enhance calcium entry, and thereby increase the discharge of neurotransmitter. To test the validity of this notion we studied the effects of two zinc chelators: (i) TPEN, which is membrane permeable and will chelate both intra- and extra-cellular zinc, and (ii) membrane-impermeable histidine, a chelator of extracellular zinc. As shown in Figures 3 and 4, similar effects were obtained with both of these drugs. Fig. 3A shows a sequence of fluorescent response peaks, corresponding to increases in internal calcium, from an isolated double cone depolarized with 30 mM KCl. The application of 250 μM TPEN together with KCl (peak 4) resulted in a marked increase in calcium entry compared to control (peak 2). This effect was reversed after washout (peak 6), and the calcium signal was further reduced when the L-type calcium channel blocker verapamil (100 μM) was added to the bath (peak 8). The corresponding calcium images are depicted in Fig. 3B, and the bar graphs in Fig. 3C show the normalized values for the corresponding peak responses in Fig. 3A, as well as the variances and significance of the results. We also tested the effects of a range of concentrations (100 – 500 μM) of TPEN with and without 100 μM verapamil. In both cases, there was little evidence of any significant dose dependence (data not shown).

The results obtained with histidine (Fig. 4) show that removal of extracellular zinc alone is sufficient to induce a similar effect as that obtained with TPEN. Using the same protocol as in Fig. 3, we observed a comparable enhancement of calcium entry after applying 500 μM histidine (Fig. 4A). Here too, the presence of an L-type calcium channel blocker (in this case nifedipine, 50 μM) eliminated the depolarization-induced calcium influx as well as the effects of histidine (peak 8). The changes in intracellular calcium corresponding to each of the time points indicated in Fig. 4A are illustrated in Fig. 4B, whereas the bar graphs of Fig. 4C present a statistical analysis of the calcium changes in response to each of the foregoing conditions. As we found with TPEN, increasing the concentration of histidine from 200 μM to 5 mM had little effect on the enhancement of calcium entry (data not shown). Note also the bar graphs of Fig. 4D, which shows that the marked reduction in the intracellular calcium response with the addition of 2 mM zinc is partially reversed by co-application of 10 mM histidine.

Based on the foregoing results, we constructed the cartoon shown in Fig. 5 to summarize the interactions between zinc, glutamate and calcium at the photoreceptor terminal, within the synaptic cleft, and among the cells of the outer plexiform layer (OPL).

4. Discussion

Owing to its unique physiology, the vertebrate photoreceptor discharges glutamate at a high rate in darkness, whereas transmitter release is reduced in graded fashion with increases in light intensity. This applies equally to zinc, which is co-packaged with glutamate in the synaptic vesicles of the photoreceptor terminal. In an earlier study, Wu and co-workers reported that very low concentrations (i.e., 5 μM) of Zn^{2+} , applied exogenously, suppressed the voltage-dependent calcium current in photoreceptor synaptic terminals (Wu et al., 1993). They concluded that since this low concentration of Zn^{2+} is able to “suppress virtually all endogenous glutamate release, the extracellular concentration of Zn^{2+} released from photoreceptors in darkness is probably lower than this level, because a substantial amount of glutamate must be released into the synaptic cleft at rest to maintain the horizontal cell dark membrane potential near -20 mV”. Interestingly, the reduced light response of horizontal cells in response to zinc application is only observed when retinas are bathed in normal calcium media (Piccolino et al., 1996). Zinc application in calcium free media, however, seems to have little blocking effect on the horizontal cell light response (Piccolino et al.,

1999b), a result attributed to the surface charge screening effect of zinc at the level of the photoreceptor terminal membrane. That aside, we should stress the fact that our experiments were performed on isolated cells, and the zinc released from their synaptic terminals was continuously washed away or diluted by the bath solution. Therefore, the concentration of endogenous zinc acting at the voltage-gated calcium channels could be significantly lower than estimated and its physiological importance could be greatly underestimated.

As we have shown, applying the zinc chelator histidine to the intact skate or zebrafish retina enhances significantly the amplitudes of the light-evoked ERG *a*- and *b*-waves, potentials derived primarily from the responses of photoreceptors and ON-bipolar cells, respectively (Redenti and Chappell, 2002; Chappell et al., 2008). This is consistent with the findings of Zhang and co-workers who have shown that ionic zinc serves to modulate signal transmission between photoreceptors and second-order neurons by suppressing AMPA receptor-mediated synaptic transmission (Zhang et al., 2002). Moreover, although the blocking effect of zinc on calcium currents has been known for some time (Winegar and Lansman, 1990; Büsselberg et al., 1992, 1994; Piccolino et al., 1996), the results obtained in the present study with both zinc chelators and calcium channel blockers suggest that the suppressive effects of endogenous zinc on calcium entry are mediated through inhibition of voltage-gated L-type calcium channels at the photoreceptor terminals.

Self-regulatory mechanisms are a typical means by which the ion channels of nerve cells modulate their own activity. This feature is of particular importance for vertebrate photoreceptors, cells that in darkness continuously discharge a neurotransmitter with potentially toxic properties (Olney et al., 1986; Izumi et al., 1995, 2003). Nevertheless, both sustained and graded release of glutamate is essential for conveying the visual message (Heidelberger, 2007). Since vesicle exocytosis at the photoreceptor synapse is mediated by calcium entry through voltage-gated channels (Corey et al., 1984; Wilkinson et al., 1996), the modulation of these channels directly affects the release of transmitter and makes them an excellent target for feedback control. In the present study, we provide experimental evidence that zinc ions are an important factor in this feedback mechanism. To circumvent uncertainties related to the quantification of zinc release, we used calcium imaging and zinc chelators to demonstrate that the discharge of endogenous zinc provides a feedback signal that is able to suppress the voltage-gated calcium channels of photoreceptors through which calcium entry triggers glutamate release (Copenhagen and Jahr, 1989; Schmitz and Witkovsky, 1997; Krizaj and Copenhagen, 2002).

A point that needs further clarification is the alternative possibility that the action of zinc is mediated via a shift of the calcium channel activation curve at the photoreceptor terminal, as proposed by Piccolino and colleagues (1996; 1999a). They suggest that the inhibiting action of divalent ions (like Zn^{2+} , Co^{2+} and Ni^{2+}) on the calcium current in the salamander and turtle photoreceptor is due to the ions' screening effects on membrane surface charge, ultimately resulting in a depolarizing shift of the activation curve of the photoreceptor's Ca^{2+} current. They further suggest that the shift in the activation curve accounts for most of what are otherwise considered the "blocking" effects of these ions on the photoreceptor calcium response. Although our experimental system did not allow us to discern between a pure channel block by zinc or a surface charge effect, it is worth noting that the surface charge hypothesis cannot fully account for the total reduction in calcium current (Cadetti et al., 2004; Piccolino et al., 1999a). It is possible, therefore, that the inhibitory effects of zinc may result from a combination of surface charge effects and a channel block. The combination of these two alternatives, and the overwhelming evidence for zinc localization in the outer retina, seems to point towards a widely conserved mechanism for zinc modulation of inward calcium currents at the synaptic region of vertebrate photoreceptors. Interestingly, it has been shown that subsequent to the initial calcium influx through voltage-

gated calcium channels, there is a release of calcium from internal stores via the activation of ryanodine receptors (Suryanarayanan and Slaughter, 2006). This mechanism probably augments the levels of internal calcium and may be important for triggering the release of neurotransmitter.

With reference to the summary diagram of Fig. 5, it seems likely that the zinc feedback mechanism is well positioned to maintain the operating level of synaptic release over a broad range of intensities. For example, in darkness when the photoreceptor is maximally depolarized, the increased amount of zinc released would be expected to reduce calcium entry, and thereby lower the discharge rate of glutamate. This process should conserve metabolic energy and protect the retina from excessive release of glutamate. Conversely, a reduction of zinc release when the cell is hyperpolarized by light will result in an increase in calcium entry, which will, in turn, increase the concomitant release of glutamate and zinc. This action will continue until the zinc concentration in the synaptic cleft is sufficient to establish a new equilibrium.

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Abbreviations

AM	acetoxymethyl
AOI	area of interest
IC₅₀	concentration giving half maximum response
OPL	outer plexiform layer
SEM	standard error of the mean
TPEN	N,N,N',N'-tetrakis(2 pyridylmethyl)ethylenediamine

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Highlights

- Exogenous zinc produced a dose-dependent reduction in calcium entry
- Zinc's effect on calcium entry is by suppression of synaptic L-type calcium channels
- Chelating endogenous zinc increased calcium entry in response to depolarization
- L-type Ca^{2+} channel blockers abolished the enhancement produced by zinc chelation
- Co-release of zinc provides a feedback mechanism that reduces glutamate release

Figure 1. Localization of calcium influx in double cones depolarized repeatedly with 30 mM KCl
(A) Trace showing the rise and fall of the calcium signal in response to a depolarizing pulse of 30 mM KCl. Each data point represents the average intensity over the area of interest (AOI), i.e., the cell soma and its terminal bulge. **(B)** Pseudo-color images of calcium dye intensity corresponding to the individual points shown in A. Scale bar = 10 μ m. Note that depolarization caused the onset of calcium influx to occur at the double cone terminal - the site of voltage-gated L-type Ca^{2+} channels (arrows). **(C)** Responses to repeated application of 30 mM KCl show a small decay in the intensity of the calcium signal. **(D)** Calcium images corresponding to the peaks and troughs in C. Scale bar = 10 μ m.

Figure 2. Application of external zinc reduces calcium entry at the cone terminal

(A) The response to depolarization (first peak, “2”) was greatly reduced by the addition of 100 μM zinc (second peak, “4”); after partial recovery (third peak, “6”), the response was further reduced (fourth peak, “7”) by the addition of the L-type calcium channel blocker, nicardipine (50 μM). (B) Calcium dye intensity images of the peaks and troughs numbered in A. Scale bar = 10 μm . (C) Bar graphs show the calcium signal intensities recorded in control (30 mM KCl), after addition of 100 μM zinc, and after addition of the L-type calcium channel blockers nicardipine (50 μM) and verapamil (100 μM) using the protocol shown in A. Although shown on the same graph, the effects of the two blocking agents were obtained from separate experiments. Error bars shown here and in subsequent figures represent $\pm$ S.E.M; asterisks indicate the significance of the differences from control: ****, $p < 0.0001$; ***, $p = 0.0002$. It is evident that the differences in the results with 100 μM zinc ($n = 9$), 50 μM Nicardipine ($n = 5$), and 100 μM Verapamil ($n = 4$) were highly significant, and that there was no significant difference between the action of the two blockers, $p = 0.98$. (D) In plotting the dose-dependent data represented by this figure, the values of the normalized peak calcium responses were corrected for decay in intensity due to bleaching. The highest (2 mM) and lowest (2 μM) concentrations of zinc inhibited $\sim 95\%$ and $\sim 9\%$ of the intensity increase observed in response to the initial depolarization-induced level of calcium entry.

Figure 3. Removing endogenous zinc increases calcium entry

(A) Application with 250 μ M TPEN enhances the calcium signal generated by depolarization (see second peak, “4”). The enhancement is reversed by removal of TPEN (third peak, “6”), and further attenuated by the addition of the L-type Ca^{2+} channel blocker verapamil (fourth peak, “8”). (B) Fluo-4 intensity images corresponding to the time points numbered in the traces shown in A; scale bar = 10 μ m. (C) Bar graphs of calcium signaling responses obtained in the sequence shown in A. Asterisks and error bars indicate the same metrics as in Fig. 2. For comparison, both blockers are shown on the same bar graph, although they were applied in separate experiments. Data show the results for 250 μ m TPEN ($n = 8$). 50 μ M nicardipine ($n=4$), and 100 μ M verapamil ($n=4$). There was no significant difference between the action of the two blockers, $p = 0.2317$, and no appreciable dose-dependent effect resulted from concentrations higher (500 μ M) or lower (100 μ M) than 250 μ M TPEN (data not shown).

Figure 4. Effects of the membrane-impermeable zinc chelator histidine

(A) Application with 500 μ M histidine (second peak, “4”) yielded results that closely resembled those obtained with TPEN. Blocking L-type calcium channels with 50 μ M nicardipine resulted in a marked reduction in calcium entry and loss of the enhancement effect of histidine. (B) Images of the calcium signals corresponding to the time points numbered in A; scale bar = 10 μ m. (C) Bar graphs depicting quantitatively the averaged data for 500 μ M histidine ($n = 18$), 50 μ M nicardipine ($n = 10$), and 100 μ M verapamil ($n=9$); there was no significant difference between the action of the two blockers. Asterisks represent the significance of the differences between control and the test drugs. (D) The inhibitory effect of 2 mM zinc is partially reversed by co-application with 10 mM histidine. Asterisks represent significant differences from control (***, $p=0.0002$; *, $p<0.05$; $n = 5$, paired t-test for both).

Figure 5. The cellular interactions mediated by zinc-related changes within the OPL

A cartoon showing the co-release of zinc and glutamate packaged within the synaptic vesicles of photoreceptor terminals, and its post-synaptic effects. In darkness, the depolarized photoreceptor releases into the extracellular space both zinc and glutamate. The latter binds to ionotropic and metabotropic glutamate receptors on second-order neurons to activate bipolar and horizontal cells. On the other hand, free zinc feeds back to the terminal, and suppresses the entry of calcium through voltage-gated Ca^{2+} channels, thereby reducing the discharge of neurotransmitter. It is likely that this feedback loop allows for the establishment of a new equilibrium of vesicle cycling at the synapse, maintains a more steady level of signaling, and regulates the tonic discharge of glutamate.