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THE EFFECTS OF ZINC DURING VISUAL ADAPTATION OF VERTEBRATE EYE

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Abstract — Zinc plays a key role in genetic expression, cell division, and growth and is essential for the function of more than 200 enzymes; effects of zinc deficiency induce many syndromes, including abnormal visual adaptation. The pigment epithelium (EP) contains high concentrations of zinc in humans and in animals and it participates in threshold elevation, visual sensitivity increment, and acceleration of rhodopsin regeration during visual adaptation. The origin of c-wave of electroretinogram(ERG) is not only pigment epithelium as shown in present research, but also other cell layers, perhaps the photoreceptors. We propose zinc as a candidate for an internal messenger which participates in signal amplification.

INTRODUCTION

Zinc is an integral part of more than 200 vital metalloenzymes and proteins, including transcription factor[1,2], hormonal receptor[3], and DNA and RNA polymerases[4], and is a cofactor for gene transcription[5] and DNA replication[6]. Since the discovery of a zinc deficiency syndrome in Iran and Egypt by Prasad and his coworkers during the early 1960's[7], diverse observations on human zinc deficiency syndromes have been discovered, such as dermatitis[8], depressed immunity[9], stunting[10,11,12], hypogonadism[13], and abnormal dark adaptation[14]. In the vertebrate retina, the retinol is converted to the active form of retinaldehyde required for rhodopsin synthesis. This conversion is mediated by the zinc metalloenzyme, alcohol dehydrogenase. Zinc deficiency has been attributed to a reduction in the rate of oxidation of retinal to retinaldehyde, cause of abnormal dark adaptation as measured by use of the ERG[15,16,17].

The experiments to be presented here were designed to test how zinc affects ERG response parameters such as thresholds, a,b,c-wave amplitudes, and recovery of sensitivity during visual adaptation; and what the molecular physiological role of the zinc from an isolated bullfrog eye might be.

MATERIALS AND METHODS

Sample preparation : The experiments were performed on the eye cup of a bullfrog. The animal was isolated in darkness for at least one hour before the eyes were excised and hemisected under dim red light. A portion of the vitreous was removed using filter paper wicks and the eye cup was quickly mounted into the modified Ussing chamber, which is located in the Faraday cage. After being filled with standard frog ringer solution, 100 % oxygen was gently blown through the chamber. The frog ringer solution, buffered to a pH level of 7.5, contained 105 m*M* NaCl, 2.5 m*M* KCl, 2 m*M* $MgCl_2$, 1 m*M* $CaCl_2$, 5 m*M* glucose, 5 m*M* $NaHCO_3$, and 10 m*M* Hepes.

Optical system : The light consisting of two light pathways provided independent computer control of "stimulus" and "background". The stimulus beam was projected straight on and the background beam was reflected by a mirror into a parallel path. Two beams were combined through a beam splitter and projected onto the preparation. Each beam from the light source(12V/100V, halogen lamp) was illuminated and passed through a 505 nm interference filter, shutter and neutral density filters. Both shutters, connected to a computer, controlled background and stimulus duration. For single stimulus flash experiments, we used 200m second of flash duration.

Electrical recording : The ERG signal was achieved with fine glass micropipettes agar bridge electrode. They were filled with 3M KCl + 3 % agar ; then gently inserted with Ag-AgCl electrode before hardening. The signal was

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† *Abbreviations* : EP, pigment epithelium; ERG, electroretinogram; ND, neutral density.

amplified with a D.C pre-amplifier(WPI DAM 50), and main-amplifier, displayed on a dual beam storage oscilloscope or recorded on video tape through AD/DA converter and digital data recorder.

Zinc concentration measurement : PE and other tissues were separately weighed and digested for 2 h at 100℃ in predetermined amounts of Nitric acid. Digests were appropriately diluted with distilled, deionized water and then were analyzed by an atomic absorption spectrophotometer (Thermo Jarrell Ash, AA- scan1).

RESULTS AND DISCUSSION

Table 1 shows the zinc concentrations of bullfrog tissues in comparision with other animal tissues. It is noteworthy that the zinc concentration of PE is about 10 times higher than any other organ of bullfrog tissues. This result suggests that zinc plays an important role in visual adaptation.

Table 1. Zinc concentrations in animal tissues(mg/kg dry weight)

	HUMAN	RAT	CALF	PIG	BULLFROG
Liver	141-245	101±13	101	150.8±12	20.6±4.6
Lung	67-86	81±3	81		11.7±2.4
Muscle	197-226	45±5	86		4.2±0.6
Heart	100	73±16			16.4±1.4
Eye					12.3±0.4
Retina	571				13.4±3.5
Choroid	562				
Ciliary body	288				
Pigment epithelium					136.0±23.6
Lens					5.4±1.9

Lack of oxygen supply produces abnormal ERG response, i.e. b-wave disappearance results in anoxia. Accordingly, enough oxygen should be supplied for recording. Time is also needed for the recovery of cell damage due to the dissection of the eye. Figure 1 shows oxygenation time dependent ERG waveform from a dark adapted retina to 200 ms, ND2 flashes. As oxygenation time increased, the standard ERG waveform became apparent. This stabilizing time was apporoximately 30 min; Thus, we performed all experiments after 30 min of oxygenation.

To investigate the effects of zinc, it is necessary to

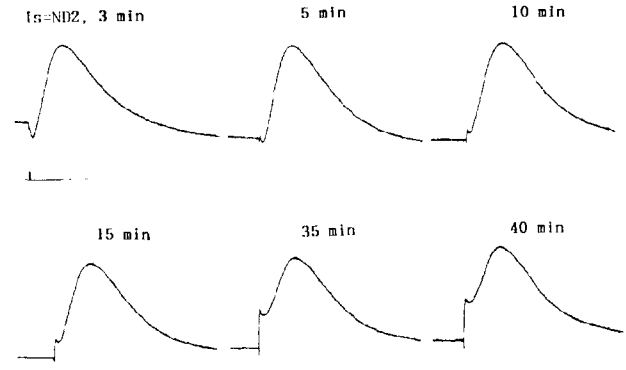

Figure 1. Oxygenation time dependent ERG waveforms. I; stimulus light intensity.

decide optimal zinc concentration for further experiments. Figure 2 shows one of the examples, which was applied 200 ms durations of flicker flash intensity in the dark adapted eye. As zinc concentration increased, the relative amplitude of b-wave increased, except at 1m*M* zinc concentration due to toxicity. Figure 3 shows statistical treatment for the whole series of data. The highest response appeared at 100μM zinc treatment in the ringer solution. But in some cases, the response decreased gradually. Therefore, we decided 1μM zinc as an optimal concentration for the experiment.

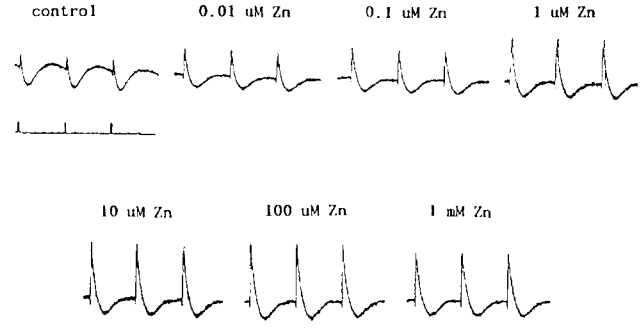

Figure 2. 200 ms flicker response increments at various zinc concentrations. Control denotes before zinc treatment.

Ideally, when a stimulus light is projected on the eye, there is steady state threshold elevation and amplitude reduction of a,b,and c-wave respectably in the presence of background light. Figure 4 shows threshold elevation at different levels of background light present, selecting a 10μV response of the b-wave as a threshold criterion. The threshold, after zinc treatment, did not increase significantly at low background intensities compared to the absence of zinc, but gradually increased in relatively high background intensities. This result indicated that the zinc is a major factor for threshold increment.

Figure 5 shows a series of the response curve at different levels of background light intensity present before and after zinc treatment. As the background intensity is decreased, each peak amplitude is bigger, while recovery of responsiveness is shortened in the zinc containing ringer solution compared to that of a zinc free solution.

As the level of adaptation changes, the operating range is shifted. The level of adaptation is set by background light. At higher levels of background light adaptation, the threshold rises, the response of ERG amplitudes reduces, and the response speeds up. Figure 6a shows b-wave response curves for the dark adapted state and in the presence of background light ND2 before and after zinc treatment separately. The b-wave increased after zinc treatment, especially at high stimulus light illumination. Figure 6b shows another example for c-wave similarly. There is c-wave increment and threshold decrement after zinc treatment.

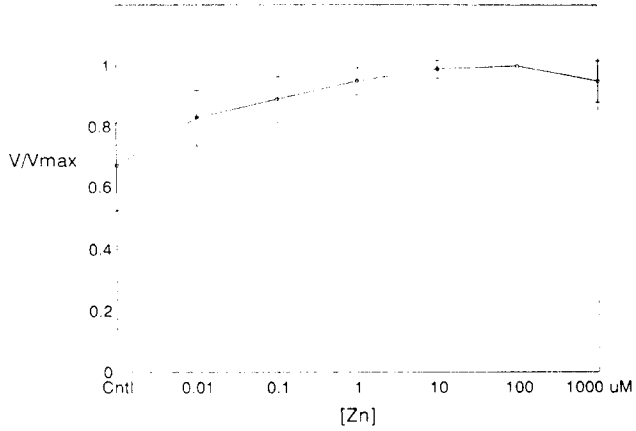

Figure 3. Relative b-wave amplitudes at various zinc concentrations. V/Vmax is the ratio of the maximum b-wave amplitude at the brightest stimulus light to the b-wave response at certain intensity.

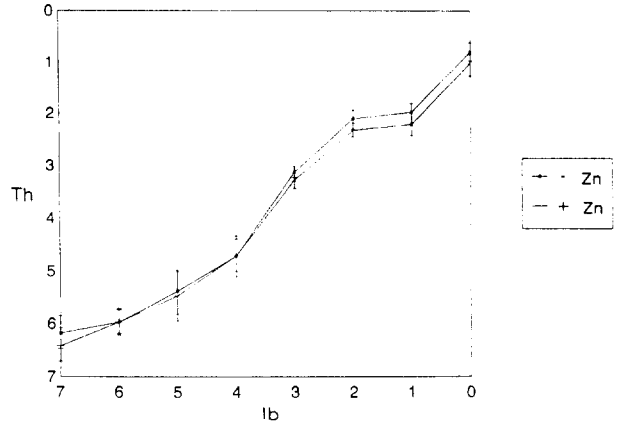

Figure 4. Threshold elevation of before and after zinc treatment. -Z, +Z, denotes before and after zinc treatment respectably. The numeric difference denotes 1 log unit of light intensity attenuation difference.

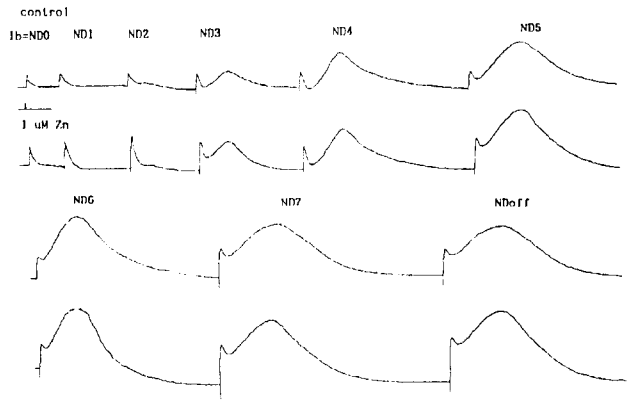

Figure 5. Comparison of ERG waveforms of stimulus light NDO at different levels of background light present before and after zinc treatment.

After a certain amount of light exposure, the ERG wave relaxes back to the base line and recovers the sensitivity which was reduced during the response. The higher stimulus of background light intensity, the longer it takes for full response recovery. This recovery correlates with the rhodopsin regeneration in the eye[21, 22, 23]. Table 2 shows the time course of b-wave threshold appearance measurement in the zinc and zinc free ringer solution after 5-min preadapting exposures of various background illumination following a weak adapting background light exposure that did not bleach a significant fraction of the visual pigment. The threshold recovery of b-wave was significantly faster compared to that of a strong adapting exposure of background light. With regard to zinc effects, there were marked accelerations of recovery after zinc treatment, especially in the strong background light.

Above results clearly indicated that zinc is a major

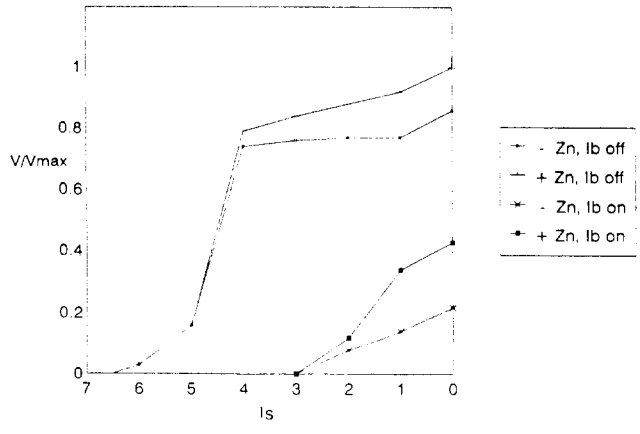

Figure 6a. B-wave response curves for the dark adapted state(upper), another for dark adapted state with a background of ND2(below).

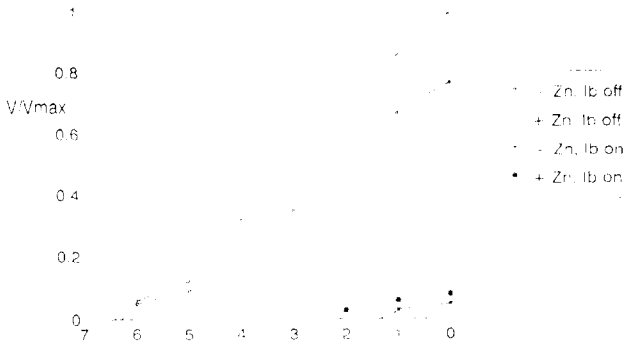

Figure 6b. C-wave response curves for the dark adapted state, another for dark adapted state with a background of ND2.

factor for threshold elevation, increment of ERG sensitivity, and acceleration of visual adaptation which is consistent with the rate of interconversion of retinal and retinaldehyde during the light and dark adaptation. Accordingly, we believe the improvement in visual adaptation in our animals treated with zinc to be due to an enhanced activity of retinal alcohol dehydrogenase.

Table 2. Time courses of dark adaptation after various 5 minute preadapting background light illumination.

Ib / Sec	ND7	ND6	ND4	ND1	NDO
Before(-Zn)	30	90	180	360	540
After (+Zn)	< 30	60	90	240	390
Difference	< 30	30	90	120	150

For ERG recording from isolated retina, a segment of the retina was peeled off, freed of pigment epithelium with the receptor side up, quickly placed on a filter paper, and mounted on the sample holder. Many experiments demonstrated that a and c-wave were not recorded from isolated retina[18, 19, 20]. These observations clearly indicated that the c-wave does not arise from the retina itself. Figure 7a shows a pair of dark adapted response curves, Figure 7b shows the response curves in the presence of background light ND4, before and after zinc treatment. Clearly, the c-wave appeared at relatively high stimulus intensities in both cases. The c-wave was more predominant in the background light illumination. These results indicated that the PE is not the only one source of c-wave origin.

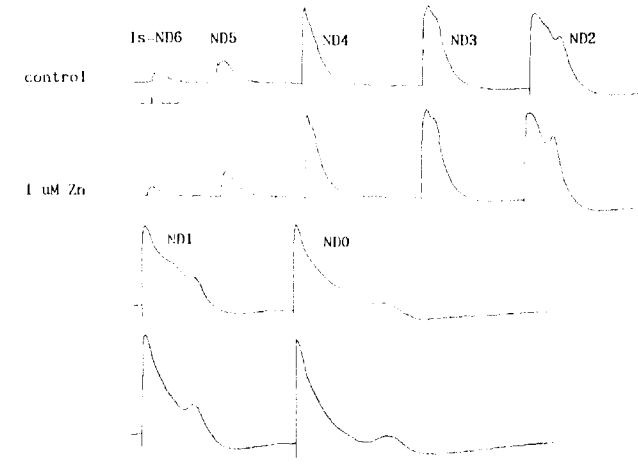

Figure 7a. Response curve in the zinc free(upper) and in the zinc treated isolated retina (below). Is; stimulus light intensity.

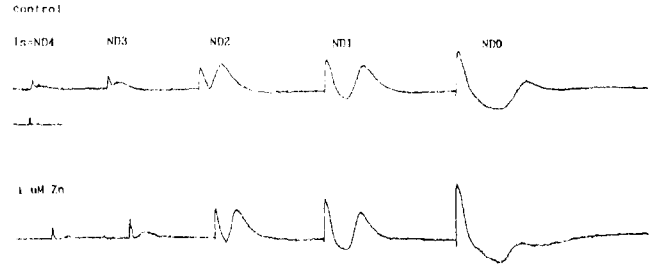

Figure 7b. Response curve in the presence of background light ND4.

CONCLUSION

The results of this study lead to three final conclusions;

1) Retinal pigment epithelium appeared to be rich in zinc, about 10 times more compared to other organ of tissue.

2) Compared to the ERG response in a zinc free ringer solution, the peak amplitude of ERG, especially b-wave, remarkably increased during the light adaptation at different levels of lash stimulus and background light intensities after 1 μM optimal concentrations of zinc treatment. On the other hand, the threshold elevated in the same manner. The recovery time of dark adaptation accelerated significantly after the zinc treatment. These results support that zinc plays important roles as a trace element for rhodopsin synthesis of visual adaptation.

3) In the case of the isolated retina, the c-wave was recorded. This result suggested that the origin of c-wave is not only pigment epithelium, but also other

cell layers. We propose that zinc plays an important role in amplifying the signal as an internal messenger, such as IP_3, Ca^{++} and cGMP.

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