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CALCIUM EFFECTS OF VISUAL ADAPTATION IN A VERTEBRATE RETINA (I)

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Abstract - Calcium has a variety of functions in neuron and muscle cells and blood clotting, especially in the visual system where dark adapted rods cotransport with Na^+ into the cell. An influx of Ca^{2+} flows out of the cell through the $Na^+ - Ca^{2+}$ exchanger.[1-4] By using a modified Ussing chamber in order to bring *in vivo* environment close, we have concluded that Ca^{2+} blocks the activity of guanylate cyclase; in consequence, having an effect on the amplitude of electroretinogram (ERG). We suggest that Ca^{2+} moves between the photoreceptor and the vitreous humor by way of certain Ca^{2+} transport mechanisms. Also, the effect of Zn^{2+} in Ca^{2+} - free ringer solution caused an elevation of amplitude in ERG and a reduction of threshold.

INTRODUCTION

There has been considerable speculation that decreased cytoplasmic Ca^{2+} concentration of the rod outer segment in the vertebrate retina may play a role in visual adaptation.[1-6] Photoexcited rhodopsin triggers an enzymatic cascade that leads to the activation of a cGMP phosphodiesterase and rapid hydrolysis of cGMP.[4-10] Recovery of the dark state requires the resynthesis of cGMP, which is catalysed by guanylate cyclase. The lowering of the cytosolic Ca^{2+} concentration following illumination is thought to be important in stimulating cyclase activities, which increases several fold of cGMP concentration. It is evident that cGMP and Ca^{2+} levels are reciprocally controlled by negative feedback.[9-16,19] These Ca^{2+} balances are controlled by the cGMP-activated cation-specific channel and $Na^+ - Ca^{2+}$ exchanger.[10,17] It is generally accepted in the present time that the visual cascade only occurs in the parts of the photoreceptor; and Ca^{2+}, which is present in the photoreceptor side, plays a key role in the process of visual adaptation. However, the above mentioned Ca^{2+} transport experiments were done with patch clamp, intracellular recording, in which the photoreceptor and sclera side of the retina were exposed to the ringer solution.[6,18] Therefore, those experiments were only limited to the study of the mechanism of Ca^{2+} transport in the photoreceptor side.

The purpose of our experiment was to study the ERG of the a -, b - wave effects in the normal ringer solution (NRS) and Ca^{2+} - free ringer solution (Ca^{2+} FRS) treated with EDTA and EGTA and to study how Ca^{2+} affects the threshold, amplitude, and regeneration time in the presence and absence of divalent cation during the light response. These experiments were performed by using a modified Ussing chamber in order to bring *in vivo* environment close.

MATERIALS AND METHODS

Dissection. A bullfrog was dark-adapted for at least 2 hours before decapitation. Dissection was performed in dim red light. The eyes were enucleated, the anterior portion cut away, and the posterior eyecup portion was mounted onto the modified Ussing chamber containing Bullfrog ringer solution: 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 buffered to a pH of 7.5.

Instrumentation. The optical system contained two light paths with interposed 505 nm interference filters, neutral density (ND) filters, and an electronic shutter. The stimulus beam was projected straight to deliver 200 msec flashes, which served as the stimulus, and the background beam to project a steady background light was reflected by a mirror into a parallel path. Two beams were combined through a beam splitter and evenly illuminated onto the preparation. The ERG was monitored with Ag-AgCl agar bridge electrodes, which were placed into the modified Ussing chamber, amplified with a DC pre-amplifier and main amplifier, and displayed on a dual beam storage oscilloscope or recorded on video tape through AD/DA converter and

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† *Abbreviations* : ERG- electroretinogram; NRS- normal ringer solution; FRS- free ringer solution; ND- neutral density

digital data recorder. There was little deterioration of ERG sensitivity, amplitude, or waveform in most preparations during the 5-6 hours of the experiments. All data were analyzed with Axotape and plotted with Sigma plot.

Ca^{2+} concentration measurement. The dark-adapted eyes were exposed to a desired level of background light for a predetermined time and then, quickly dropped into liquid nitrogen. The frozen eye balls were peeled of the scelera with a razor blade to extract the vitreous humor, then separately, the volume was measured, and was digested for 2 hours at 100°C in predetermined amounts of nitric acid. A Thermo Jarrell Ash atomic absorption spectrophotometer was used for Ca^{2+} concentration measurement.

RESULTS AND DISCUSSION

ERG changes in NRS treated with EDTA. Fig. 1 shows the relative amplitude of a – and b – wave and a – wave after being treated with EDTA during light response.

The x-axis represents NRS, EDTA-treated NRS, and various time periods after treatment; the left y-axis represents the relative amplitude of a – and b – wave; and the right y-axis represents the relative amplitude of a – wave. We used ND 1 as a light source and the larger the ND intensity the lower the log unit was. Relative ampltitude occurred in EDTA-treated NRS after 5 and 10 minutes ; however, in all following time periods, that amplitude decreased. This decrease occurred because EDTA damaged or destroyed the retina membrane and because of the chelation of divalent cation.

EDTA-treated Mg^{2+} FRS. To find out the reasons behind the amplitude when NRS was treated with EDTA, we eliminated divalent cation in NRS one by one. In order to chelate Ca^{2+} and other metal ions in the Mg^{2+} FRS, 2 m*M* EDTA was added and those results are plotted in Fig. 2, which shows the relative amplitude following various time periods.

We observed that there was no significant change when Mg^{2+} FRS replaced NRS, but when EDTA was added to this new solution, the amplitude elevation had a similar tendency of when EDTA was added to NRS. Thus, Mg^{2+} had no effect of amplitude elevation after EDTA treatment.

EDTA-treated Ca^{2+} FRS. To chelate other divalent metal ions, we used 3 m*M* EDTA to Ca^{2+} FRS which contains the same concentration (2 m*M*) of $MgCl_2$. Fig. 3 shows the relative amplitude after treatment of EDTA in NRS.

We observed an increase in the relative amplitude when Ca^{2+} FRS replaced NRS. The increase in a – and b – wave of Ca^{2+} FRS decreased again because of the addition of EDTA, but the amplitude elevation due to EDTA that was shown in previous experiments did not appear. The reason for this amplitude decrease after EDTA treatment was the chelation of divalent ions and cell damage by the EDTA.

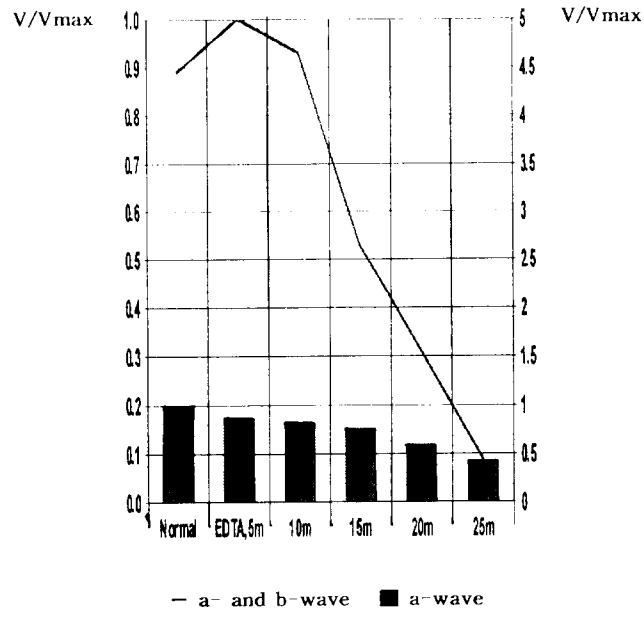

Figure 1. Relative amplitude (V/V max) of a – and b – wave and a – wave after adding EDTA-treated NRS (I_s= ND 1).
I_s : Stimulus light intensity

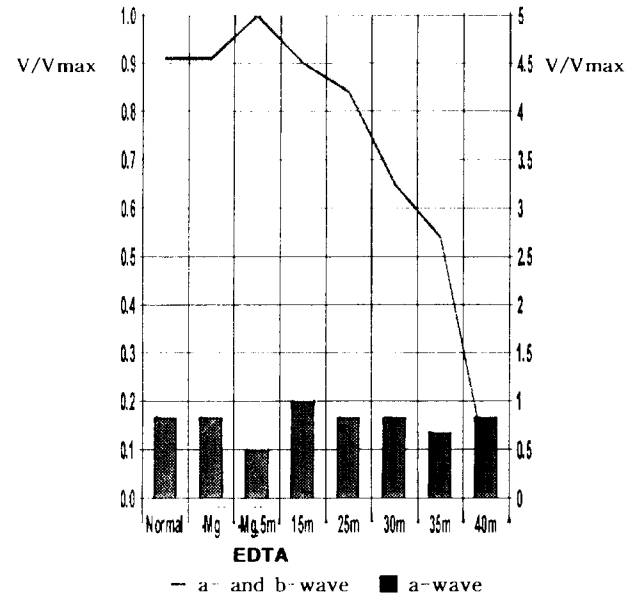

Figure 2. Relative amplitude of a – and b – wave and a – wave after adding EDTA-treated Mg^{2+} FRS (Is= ND 1).

EGTA treatment in NRS. In the previous experiments, we came to know that Ca^{2+} affects the ERG amplitude. So, we used EGTA, which chelates only Ca^{2+}, to find out the exact function of Ca^{2+}. Fig. 4 shows the relative amplitude of EGTA-treated NRS.

The x-axis represents NRS, EGTA-treated NRS, and various time periods after treatment. Amplitude elevation appeared because of the chelation of Ca^{2+} when

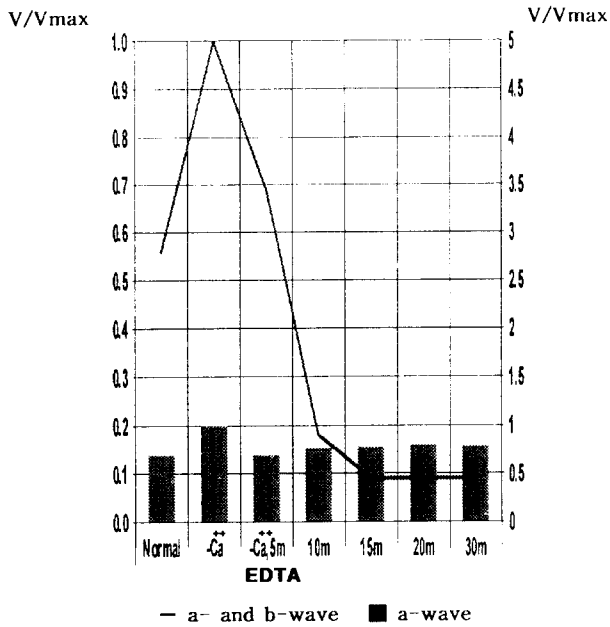

Figure 3. Relative amplitude of a – and b – wave and a – wave after adding EDTA-treated Ca^{2+} FRS (I_s= ND 1).

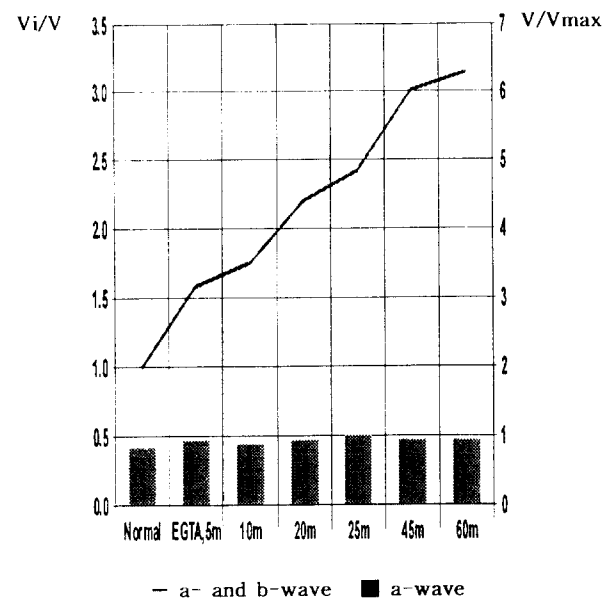

Figure 4. Relative amplitude (V_i/V) of a – and b – wave and a – wave after adding EGTA-treated NRS.

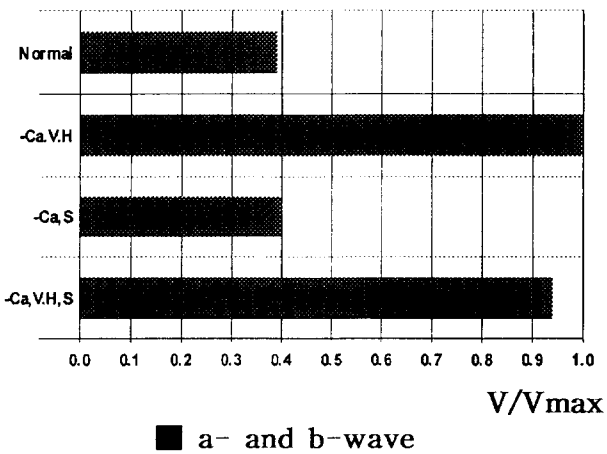

Figure 5. Ca^{2+} effect depending on eye cup position. V.H. : vitreous humor side, S : sclera side

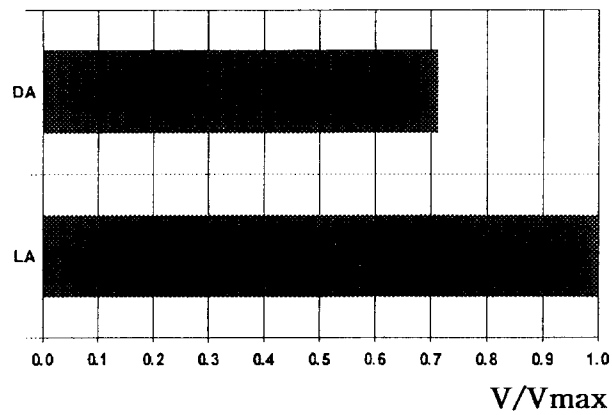

Figure 6. Ca^{2+} concentration in the vitreous humor during dark adaptation (DA) and light adaptation (LA).

EGTA was added to the NRS; also, there was no significant change in a – wave, but an increase in b – wave appeared relatively.

Comparison of ERG among vitreous humor side, scelera side, and both sides. We conducted experiments to find out where Ca^{2+} has the most influence among the vitreous humor, sclera, and both sides. As shown in Fig. 5, in the case where Ca^{2+} FRS was added to the vitreous humor side, that part of the retina had the most influence.

However, in the case of adding Ca^{2+} FRS to the scelera side and both sides, there was not a significant effect as there was in the vitreous humor side. Therefore, all following experiments involved comparison between Ca^{2+} FRS and NRS in only the vitreous humor side.

Ca^{2+} concentration differences between light adaptation and dark adaptation evoked by light stimulation in vitreous humor. Fig. 6 shows Ca^{2+} concentration in the vitreous humor between light and dark adaptation by light stimulation that evoked transportation of Ca^{2+} which came from the rhodopsin outer segment.

We used approximately 100 eyecups and all data was processed statistically. The x-axis represents the relative value of dark adaptation to light adaptation. The y-axis represents dark adaptation and light adaptation. Ca^{2+} concentration in the vitreous humor during light adaptation was obviously higher than during dark adaptation.

Ca^{2+} concentration that has the greatest effect to the retina. Fig. 7 shows the relative a – and b – wave amplitude change which was aroused by the exchanges

of different Ca^{2+} concentration in the NRS.

The x-axis represents the difference of Ca^{2+} concentration by unit of m*M*. The control NRS contained 1 m*M* Ca^{2+} and as shown in Fig. 7, the control had a bigger difference in relative amplitude than in Ca^{2+} FRS. In the case of the isolated retina, the same tendency occurred. It seemed that the cell showed Ca^{2+} toxicity when we added 100 m*M* Ca^{2+}.

ERG comparison between Ca^{2+} FRS and NRS. To experiment what effect Ca^{2+} has in light adaptation between NRS and Ca^{2+} FRS, first, we illuminated background and stimulus light in which each light had a different intensity. Then, we recorded ERG changes between NRS and Ca^{2+} FRS. For example, Fig. 8 shows

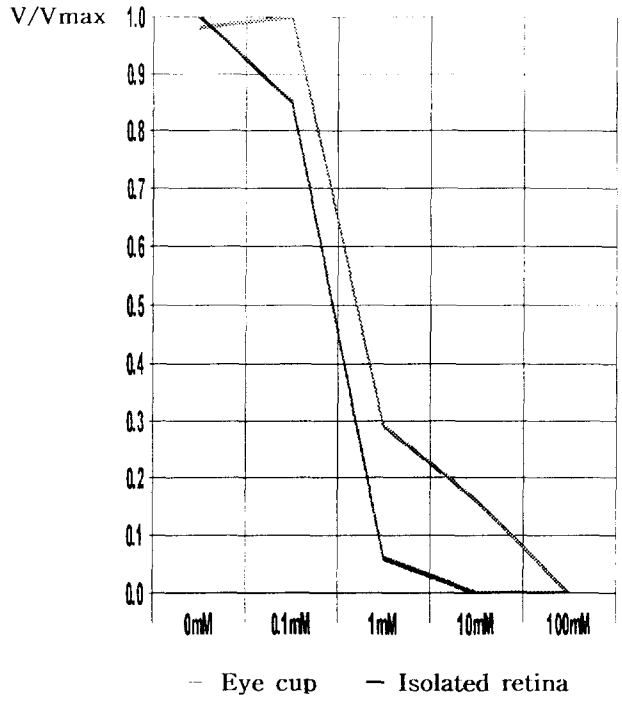

Figure 7. Relative amplitude of a – wave depending on Ca^{2+} concentration

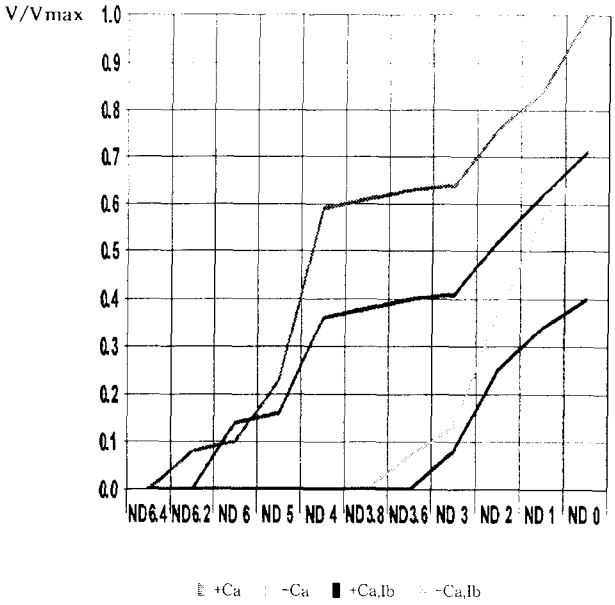

Figure 8. Relative amplitude of a – and b – wave and ND 3 background light.

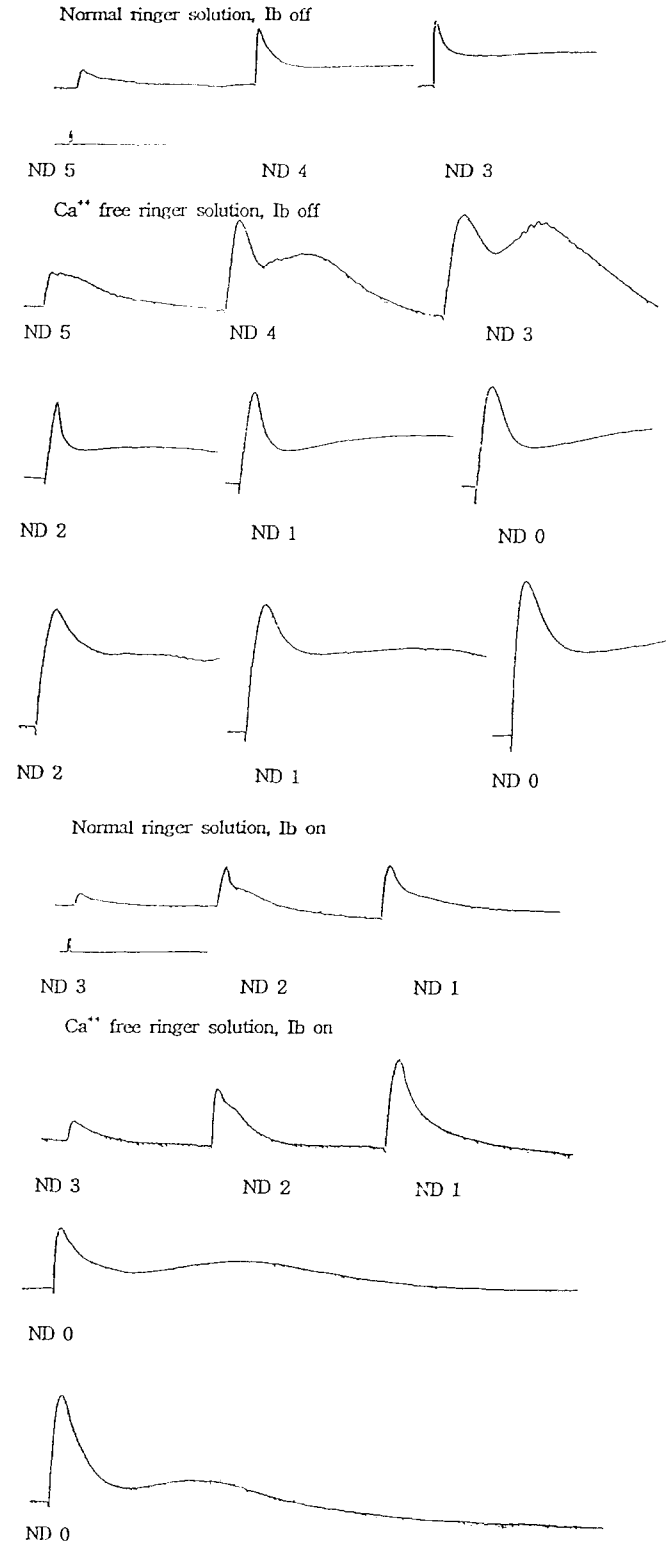

Figure 9. Comparison of ERG waveform at ND 3 background light at different levels of stimulus light between NRS and Ca^{2+} FRS

the comparison between NRS and Ca^{2+} FRS at ND 3 background light.

The two upper lines are NRS and Ca^{2+} FRS without background light and the bottom two lines are NRS and Ca^{2+} FRS with ND 3 background light. In the absence of background light, the relative amplitude wave of Ca^{2+} FRS increased more than that of NRS. In the presence of background light, the same increasing tendency also appeared. However, the threshold change reduced in both cases. Fig. 9 shows the ERG wave form in the case of the presence and absence of background light.

Regeneration time comparison between NRS and Ca^{2+} FRS. At first, after obtaining the threshold from each of NRS and Ca^{2+} FRS, we exposed them to the background light and recorded the threshold appearing time after turning off the background light as shown in Fig. 10. The x-axis represents the light intensity and the y-axis represents the relative value of the threshold appearing time.

The stronger the background light was, the slower the regeneration time became, and also, Ca^{2+} FRS had a shorter regeneration time than that of NRS. These results suggest that Ca^{2+} has an effect on regeneration time evoked by light stimulus.

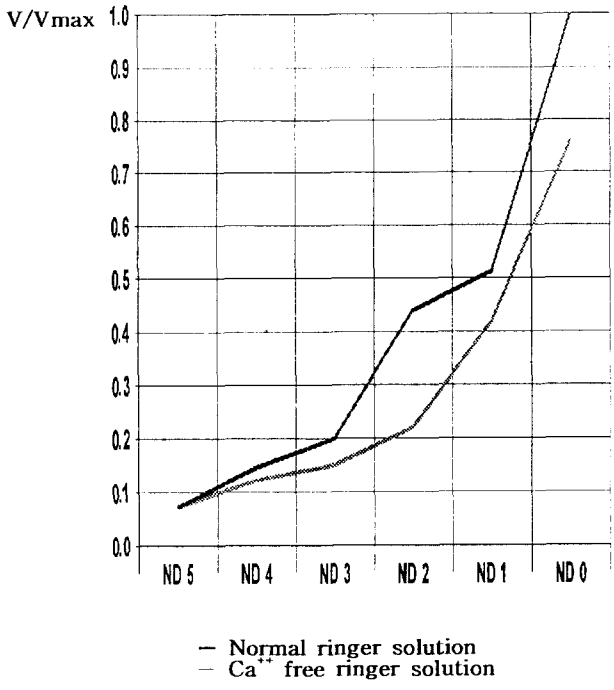

Figure 10. Regeneration time in NRS and Ca^{2+} FRS.

ERG change in Zn^{2+}-treated Ca^{2+} FRS. We added Zn^{2+}, which plays an important role in the visual system, to the Ca^{2+} FRS and studied the a – and b – wave and threshold change compared with NRS. Fig. 10 shows each threshold and amplitude among NRS, Ca^{2+} FRS, and Zn^{2+}-treated Ca^{2+} FRS.

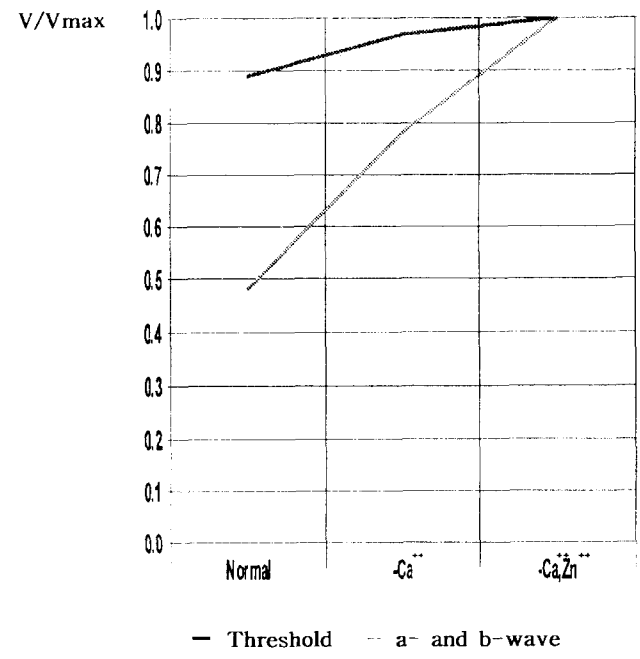

Figure 11. Relative amplitude and threshold in NRS, Ca^{2+} FRS, and Zn^{2+}-treated Ca^{2+} FRS
upper line : threshold lower line : amplitude

We used ND 1 as a stimulus light; the x-axis represents the kind of solution; and the y-axis represents the relative amplitude and threshold of each a – and b – wave. There was a threshold increase due to the exchange of NRS to Ca^{2+} FRS, but there was no significant increase when Zn^{2+}-treated Ca^{2+} FRS solution replaced Ca^{2+} FRS. Instead, the amplitude increased remarkably. So, according to the above results, Zn^{2+}, as a divalent cation, which elevates ERG amplitude and reduces the threshold when it is added to Ca^{2+} FRS, plays an important role in visual sensitivity.

CONCLUSION

The results of this study lead to the following conclusions:

1) When NRS was treated with EDTA, evidence of amplitude elevation appeared. This phenomenon was due to the chelation of Ca^{2+}. This result confirmed that only chelated Ca^{++} using EGTA caused an elevation in a – and b – wave amplitude.
2) The location where Ca^{2+} had the highest effect on amplitude was the vitreous humor side of an eyecup.
3) After measuring the Ca^{2+} concentration in the vitreous humor, during light adaptation, the concentration of Ca^{2+} in the vitreous humor was higher than that in dark adaptation. So, we suggest that certain Ca^{2+} transport mechanisms exist between the photoreceptor and the vitreous humor side.
4) We verified that Ca^{2+} FRS has a greater effect on

amplitude elevation than that of any concentration of Ca^{2+} in the eye cup and the isolated retina.

5) In a variety of intensities in each the background light and stimulus light, an elevation of amplitude in ERG appeared. This elevation occurred when using Ca^{2+} FRS which was higher than when NRS was treated. Also, the threshold was reduced.
6) The regeneration time accelerated when Ca^{2+} FRS was used in dark adaptation.
7) Ca^{2+} FRS treated with Zn^{2+} had a higher elevation of ERG than that of NRS or Ca^{2+} FRS. In addition, the threshold was reduced. It means that because Zn^{2+} has an effect on retinol dehydrogenase, visual adaptation was accelerated.

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