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THE NEW FINDING OF A LIGHT DEPENDENT Ca^{2+} CHANNEL AND Na^{+} – Ca^{2+} EXCHANGER IN THE VERTEBRATE RETINA (II)

YUN SOOK KIM, HYUK JUNG, CHANG SUCK PARK, SUK HYANG WOO, HELEN SON, HYUN JUNG KIM, and YOU YOUNG KIM*
Department of Biochemistry, College of Natural Sciences Kyungpook National University, Taegu, 702-701, Korea

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Abstract - Calcium modulates the activity of guanylate cyclase and plays a key role in dark and light adaptation in the visual system. We have measured the Ca^{2+}, K^{+} and Na^{+} concentration in dark and light adapted bullfrog's (*Rana catesbeiana*) vitreous humor by using the atomic absorption spectrophotometer. The calcium concentration of the light adapted bullfrog's vitreous humor was higher than that of the dark adapted bullfrog's vitreous humor. This means that ion activity between the photoreceptor and vitreous humor side is light dependent and we have found that a Ca^{2+} channel and Na^{+} – Ca^{2+} exchanger exist in the vitreous humor.

INTRODUCTION

The vertebrate visual system can operate over a large range of light intensities. This is possible in part because the sensitivity of photoreceptors decreases approximately in inverse proportion to the background light intensity.[1-3] This process, called photoreceptor light adaptation, is known to be mediated by a diffusible intracellular messenger,[4-6] but the identity of the messenger is still unclear. The decreased internal Ca^{2+} concentration may play a role in light adaption.[7-11] In darkness a steady Ca^{2+} influx into the photoreceptor outer segment through the light dependent channels[12-14] is balanced by an equal efflux of Ca^{2+} by Na^{+} – Ca^{2+} exchanger.[15-17] Visual excitation in retinal rod cells is mediated by a cascade that leads to the amplified hydrolysis of cyclic GMP (cGMP) and the consequent closure of light sensitive channels in the plasma membrane.[18,19] Light closes the channels and the continued extrusion of Ca^{2+} produces a decrease in internal Ca^{2+} concentration.[17] The guanylate cyclase activity of bovine rod outer segment (ROS) membranes is highly dependent on the concentration of Ca^{2+}.[19] The highly cooperative activation of guanylate cyclase by the light-induced lowering of internal Ca^{2+} concentration is likely to be a key event in restoring the dark current after excitation.[18,19] Changes in internal Ca^{2+} concentration have been shown to modulate the synthesis and hydrolysis of cGMP by guanylate cyclase[14,20] and phosphodiesterase.[10,21] The transducin, activated by photolyzed rhodopsin, may lead to increased activity of both phosphodiesterase and guanylate cyclase to mediate the desensitization (by reducing the dark current) and the faster recovery of the light adapted response.

The purpose of our experiment was to identify what kinds of light dependent transport systems exist in the vitreous humor side during light adaption.

MATERIALS AND METHODS

The experiments were performed using the same materials and methods as described in the previous paper. Please refer to the paper on Ca^{2+} effects on visual adaptation in a vertebrate retina (I)[22].

RESULTS AND DISCUSSION

Fig. 1. shows Ca^{2+} concentration differences in the vitreous humor from a light adapted retina to 5 minutes flashes of various intensities.

The abscisa represents light adaptation, the neutral density (ND) attenuation light, and dark adaptation. The ordinate is the relative Ca^{2+} concentration peak of dark adaptation to each of the stimulus light intensities. As the stimulus light intensity increased, the Ca^{2+} concentration in the vitreous humor became higher. These results suggest that Ca^{2+} moves through a certain light dependent transport system which might exists in the vitreous humor side during the courses of light adaptation.

Fig. 2 shows Na^{+} and K^{+} concentration differences between dark and light adaptation by measuring the same

* To whom correspondence should be addressed.
† *Abbreviations* : ERG – electroretinogram

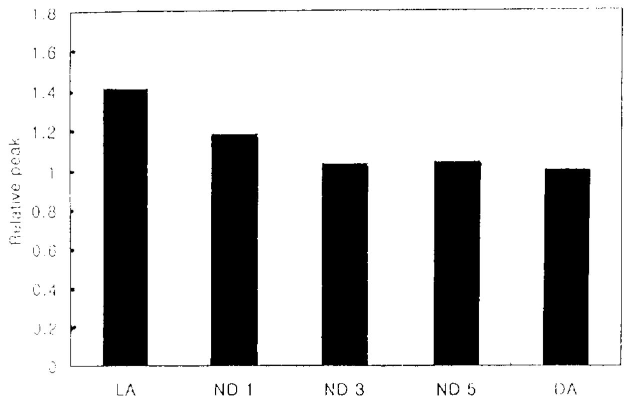

Figure 1. Ca^{2+} concentration differences in the vitreous humer depending on stimulus light intesity.

Table 1. The compositon of Bullfrog ringer solution

Concentration	Chemical agent
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*	$NaHCO_3$
10 m*M*	HEPES

pH = 7.5

method as shown in Fig. 1. K^+ and Na^+ concentration in the vitreous humor during light adaptation was obviously higher than during dark adaptation.

Based on the above results, we can accept the proposition that the Ca^{2+} channel and $Na^+ - Ca^{2+}$ exchanger is present in the side of the vitreous side of the retinal membrane.

We treated the Ca^{2+} channel blocker (Ni^{2+}, CO^{2+}, Cd^{2+}, Mn^{2+}, Mg^{2+}) as a method of preventing Ca^{2+} entry to the vitreous humor side in order to confirm the existence of Ca^{2+} channels. Mg^{2+} free ringer solution was used for the following experiments because the Bullfrog ringer solution contains the Ca^{2+} channel blocking Mg^{2+}. The composition of the Bullfrog ringer solution we used in experiments is shown in Table 1 as a reference.

Comparison of the relative ERG a− and b−wave amplitude peak after Mg^{2+} free ringer solution was treated with Ca^{2+} channel blocking Co^{2+} (2 m*M* $CoCl_2$) is shown in Fig. 3. The illuminated light intensity was ND 1.

The abscisa represents the sorts of solution and the ordinate is the relative peak of a− and b−wave. The (+) and (−) denotes removal and addition of divalent cation in the ringer solution respectably. The a− and b−wave amplitude decreased remarkably after Co^{2+} treatment compared to the ERG response in Mg^{2+} free or Mg^{2+}, Ca^{2+} free ringer solution. Fig. 4 shows the examples of typical ERG wave form exposed to ND 1 stimulus light intensity

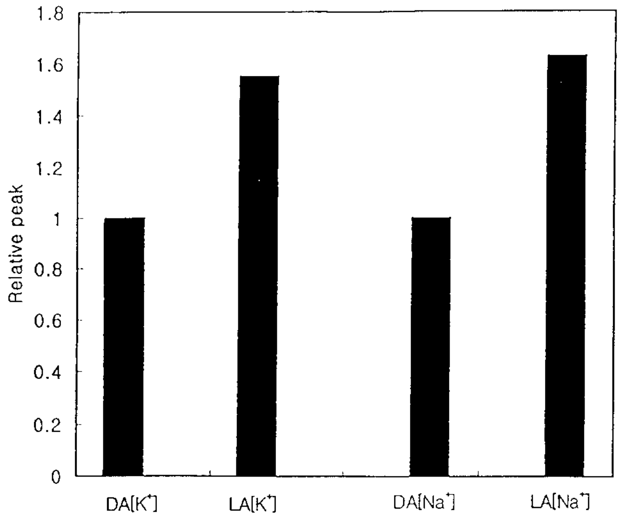

Figure 2. K^+ and Na^+ concentration in the vitreous humer during light and dark adaptation DA [K^+] : K^+ concentration during dark adaptation, LA [K^+] : K^+ concentration during light adaptation, DA [Na^+] : Na^+ concentration during dark adaptation, LA [Na^+] : Na^+ concentration during light adaptation

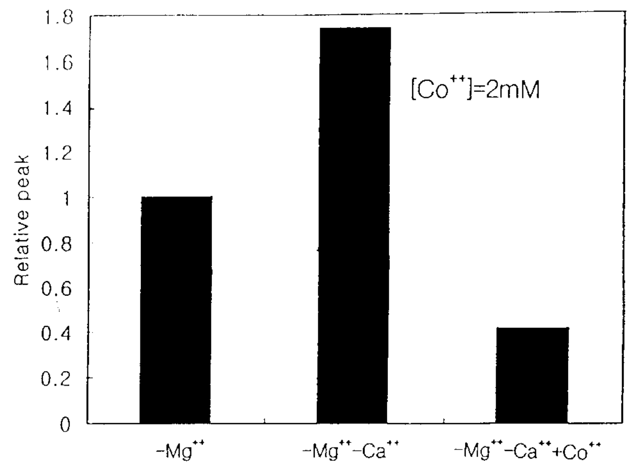

Figure 3. The relative ERG ab−wave peak after Mg^{2+} free ringer solution was treated with Ca^{2+} channel blocking $CoCl_2$. $-Mg^{2+}$: Mg^{2+} free ringer solution, $-Mg^{2+}-Ca^{2+}$: Mg^{2+}, Ca^{2+} free ringer solution, $-Mg^{2+}-Ca^{2+}+Co^{2+}$: Mg^{2+}, Ca^{2+} free ringer solution was treated with $CoCl_2$

after Mg^{2+} free ringer solution was each treated with Cd^{2+} (2 m*M* $CdCl_2$) and Ni (2 m*M* $NiCl_2$).

There was no comparable change in the a−wave, but the b−wave was suppressed. The a−wave originated from the photoreceptor. Accordingly, these results suggest that even though the photoreceptor performed its function ie., during illumination, when the light sensitive channels are closed and inward leak of Ca^{2+} is thereby suppressed, the $Na^+ - Ca^{2+}$ exchanger continues to operate and the free Ca^{2+} concentration falls to a lower level, the

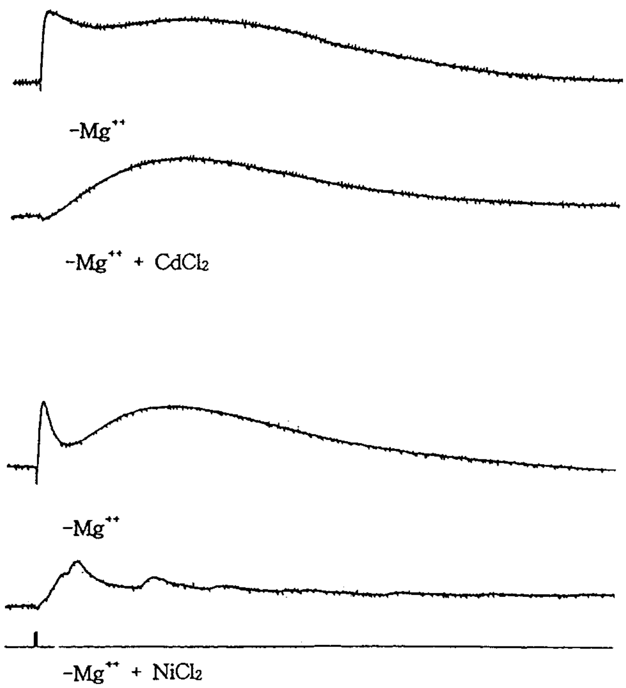

Figure 4. The ERG waveform after Mg^{2+} free ringer solution was each treated with $CdCl_2$, and $NiCl_2$

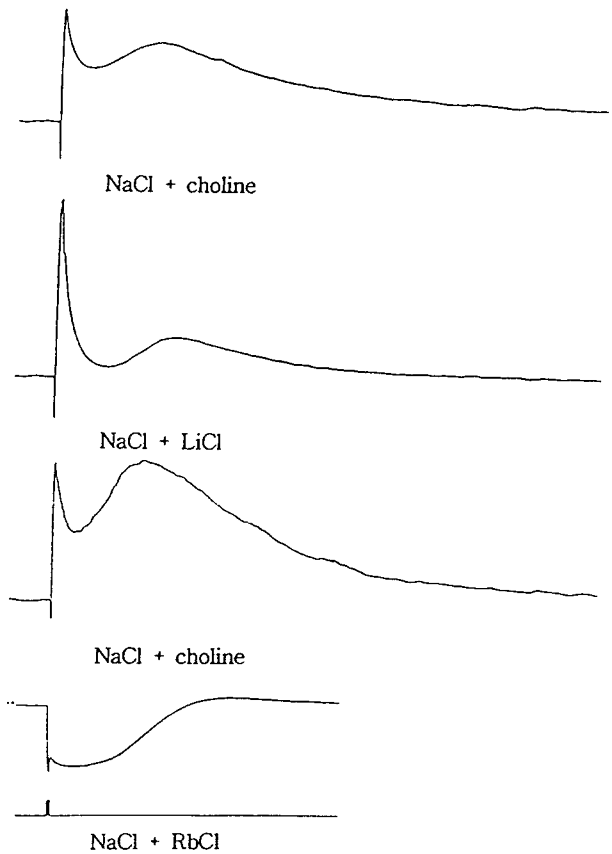

Figure 5. The ERG waveform after each LiCl and RbCl was treated in place of choline

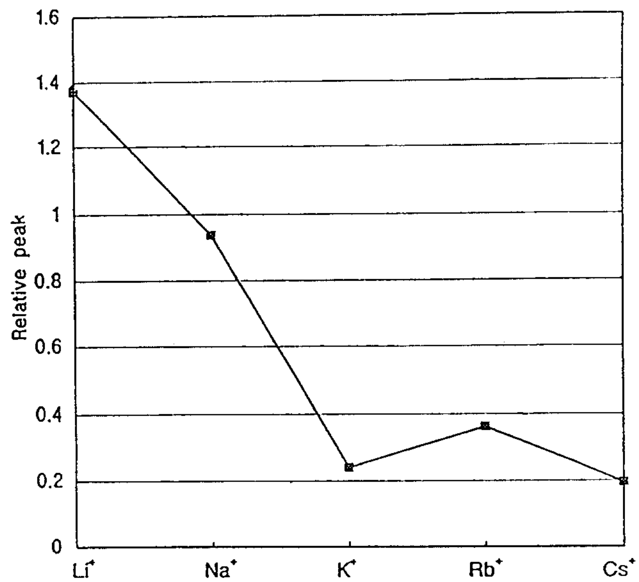

Figure 6. ab – wave amplitudes after monovalent cation treatment

Ca^{2+} channel in the vitreous membrane was blocked after blocker treatment. This means that the Ca^{2+} channel exists in the vitreous humor side membrane.

From previous data, K^+ and Na^+ concentration in the vitreous humor is higher during light adaptation than during dark adaptation, which implies those ions move through the $Na^+ - Ca^{2+}$ exchanger. We treated $Na^+ - Ca^{2+}$ exchanger blocker and activator to the vitreous humor side membrane in order to prove the existence of the exchanger. The NaCl concentration was reduced by half (52.5 m*M* NaCl) and replaced it with choline (52.5 m*M*), which has no effect on ERG waveform and a – and b – wave amplitude.

Fig. 5 shows the example of ERG waveform after each LiCl and RbCl was treated in replace of choline. After treating LiCl, being an activator of $Na^+ - Ca^{2+}$ exchanger, the b – wave amplitude increased, but there was no comparable change of a-wave. After treating RbCl, being a blocker of $Na^+ - Ca^{2+}$ exchanger, the b – wave amplitude decreased. In this case, a – wave emerged to hyperpolarize b – wave. The a – and b – wave amplitudes due to the replacement of the NaCl concentration by half with the monovalent cation (Li^+, Na^+, K^+, Rb^+, Cs^+) are plotted in Fig. 6.

The above results are similar to those the experiment to prove of the existence of $Na^+ - Ca^{2+}$ exchanger in the rod outer segment. So, this means that a $Na^+ - Ca^{2+}$ exchanger exists in the vitreous humor side.

CONCLUSION

The results of this study lead to the following conclusions:

1) Ca^{2+} concentration in the vitreous humor increased as

the stimulus light intensity became higher. K^+ and Na^+ concentration in the vitreous humor was higher during light adaptation than during dark adaptation.

2) When we treated the vitreous humor with Ca^{2+} channel blocker (Ni^{2+}, CO^{2+}, Cd^{2+}, Mn^{2+}, and Mg^{2+}), during light adaptation, there was no comparable change in the a – wave, but the b – wave was suppressed. Even though the photoreceptor performed its function, because of the change in ionic concentration between the photoreceptor and vitreous humor, the b – wave originating from the neuron cell (bipolar cell, ganglion cell, horizontal cell, amacrine cell) and non-neuron cell (Mullar cell) was changed. This means that a Ca^{2+} channel exists in the vitreous humor side.

3) We reduced the NaCl concentration by half and replaced it with Li^+, Na^+, K^+, Rb^+, and Cs^+. Then, when we treated it to the vitreous humor, the b – wave was suppressed or hyperpolarized. There was no change in the a – wave, so this means that an exchanger exists in the vitreous humor side.

From these results, we have concluded that a light dependent Ca^{2+} channel and $Na^+ - Ca^{2+}$exchanger exist in the vitreous humor side of vertebrate eye.

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