IMPORTANCE OF CALCIUM AND MAGNESIUM IONS FOR POSTEXCITATORY HYPERSENSITIVITY IN THE JUMPING SPIDER (*MENEMERUS*) EYE

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SUMMARY

Hypersensitivity of the anterior median eye of the jumping spider *Menemerus confusus*, following illumination, was unaffected by the concentration of Na^+ or K^+, was depressed by increase of Ca^{2+} concentration, and was dependent upon the presence of Mg^{2+}. Sensitivity of the dark-adapted eye was also inversely related to Ca^{2+} concentration, but to a lesser extent.

INTRODUCTION

Photoreceptor cells of the anterior median eye of the jumping spider *Menemerus* are more sensitive for a brief period following illumination than they are during complete dark adaptation (Yamashita & Tateda, 1976). It was suggested that such hypersensitivity may be related to the ionic composition of the saline, since calcium ions are important in controlling the sensitivity of photoreceptor cells in other arthropods (Lisman & Brown, 1972, 1975; Brown & Lisman, 1972, 1975; Brown & Blinks, 1974; Fein & Lisman, 1975; Bader, Baumann & Bertrand, 1976; Hanani & Hillman, 1976; Fein & Charlton, 1977; Brown, Brown & Pinto, 1977; Lisman & Strong, 1979).

In the experiments reported here, the effects of the four major cations on hypersensitivity in *Menemerus* have been studied.

MATERIALS AND METHODS

Jumping spiders *Menemerus confusus* Boes et Str. were used throughout this study. Preparation and recording methods were similar to those described previously (Yamashita & Tateda, 1976). Electroretinograms (ERGs) were recorded from the anterior median eyes with glass pipette microelectrodes or suction electrodes. Two 6–8 V tungsten lamps (lamp I, II) placed side by side were used for white light stimulation. Lamp I was used as the control light and the test light, and lamp II was used as the conditioning light. Initially, the control light, serving also as the test light, was presented. This was followed by the conditioning light and after various time intervals by a test light (Fig. 1A). The control light was used on the completely dark-adapted eye. In some cases, the conditioning light was used also for background
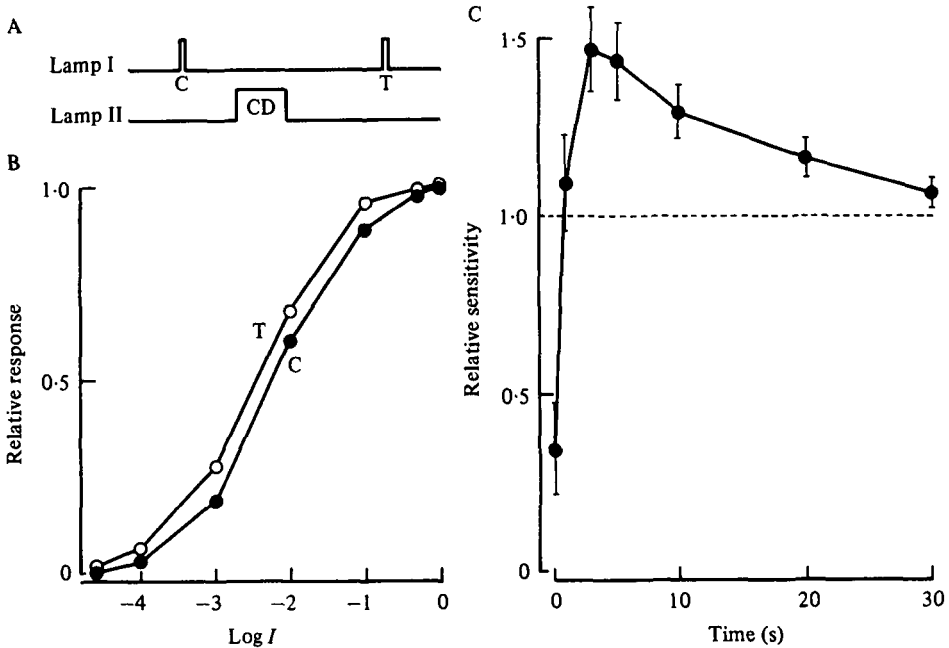
Fig. 1. (A) Schematic drawing of the control stimulus (C) given by lamp I, conditioning stimulus (CD) given by lamp II and test stimulus (T) given by lamp I. (B) ERG intensity–response relations obtained during the dark (closed circles) and 5 s after (open circles) the cessation of a 1 s duration CD. The CD was the same throughout. The saturated value of the intensity–response curve for the dark-adapted eye is set at 1·0. The relative response to each light stimulus obtained from one experiment is plotted against the relative intensity. (C) Changes of sensitivity following a CD of 1 s duration. Relative sensitivity obtained from six experiments is plotted against time after the end of CD. Vertical bars indicate the size of the standard deviations.

illumination. The composition of the normal physiological saline was as follows: NaCl, 217 mM; KCl, 5 mM; $CaCl_2$, 4 mM; $MgCl_2$, 1·1 mM; $NaHCO_3$, 3 mM (Rathmayer, 1965). Low-Na^+ salines containing 112 mM, 46 mM and 3 mM-Na^+ were made by replacing NaCl with equimolar choline chloride, and K^+-free saline by replacing KCl with choline chloride. Low-Ca^{2+} saline (0·1 mM-Ca^{2+}) was made by replacing $CaCl_2$ with equivalent NaCl, and high-Ca^{2+} saline (10 mM Ca^{2+}) by replacing NaCl with $CaCl_2$. Mg^{2+}-free saline was made by replacing $MgCl_2$ with equivalent NaCl. After a change in the perfusing solution, a period of 3–10 min was usually required to establish the steady-state effects of the new solution upon the eye's light response. Reported results were obtained repeatedly under these steady-state conditions.

RESULTS

Hypersensitivity in normal saline

ERG intensity–response curves to white light stimuli were obtained from completely dark-adapted eyes and from eyes 5 s after a constant conditioning stimulus of 1 s duration (Fig. 1 B). The intensity–response curves obtained before and af
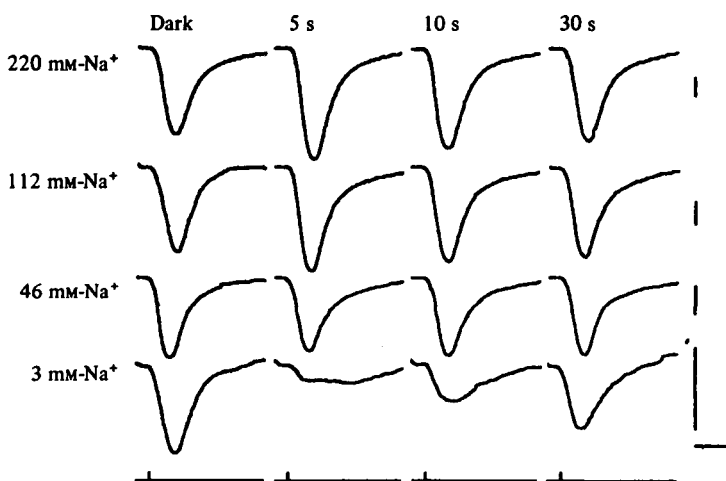
Fig. 2. Effects of decreased sodium concentration of the external saline on ERG light responses before and after a CD of 1 s duration. C (Fig. 1 A) was presented to the dark-adapted eye and T was presented 5, 10 and 30 s after the end of CD. Calibrations: 150 μV, 0.1 s.

conditioning stimuli were almost parallel; any one of them could be superimposed on the other by a shift along the abscissa.

Sensitivity was defined as the reciprocal of the relative intensity required to elicit a response amplitude 50% that of the saturated response, and the sensitivity of the completely dark-adapted eye was taken as 1.0. After the conditioning stimulus, relative sensitivity was initially very low but then increased rapidly above the dark-adapted level and fell back slowly (Fig. 1 C). In fresh preparations, maximum relative sensitivity was about 1.5, and occurred 3–5 s following the end of illumination.

Effects of sodium and potassium ions

ERG light responses before and 5 s, 10 s and 30 s after a constant conditioning stimulus were obtained in normal and in low-Na$^+$ saline (Fig. 2). In normal saline (220 mM-Na$^+$) and in 112 mM-Na$^+$ saline, the amplitude of the responses increased after the conditioning stimulus. In 46 mM-Na$^+$ saline, the response showed little increase after the conditioning stimulus, and in 3 mM-Na$^+$ saline, it markedly decreased.

In 46 mM and in 3 mM-Na$^+$ saline, the intensity–response function for the dark-adapted eye and that for the eye 5 s after the cessation of the conditioning stimulus show that the saturated values markedly decreased after illumination compared with those before illumination (Fig. 3). Note that sensitivities increased after the conditioning stimulus at both reduced Na$^+$ levels. The relative sensitivity 5 s after conditioning was 1.51 in 46 mM-Na$^+$ saline and 1.31 in 3 mM-Na$^+$ saline. These values were approximately the same as those in normal saline (cf. Fig. 1).

In 112 mM-Na$^+$ saline both the intensity–response curve during the dark and the change in sensitivity after illumination were almost identical with those in normal saline (Fig. 4). Therefore, it is unlikely that Na$^+$ plays a significant role in the hyper-
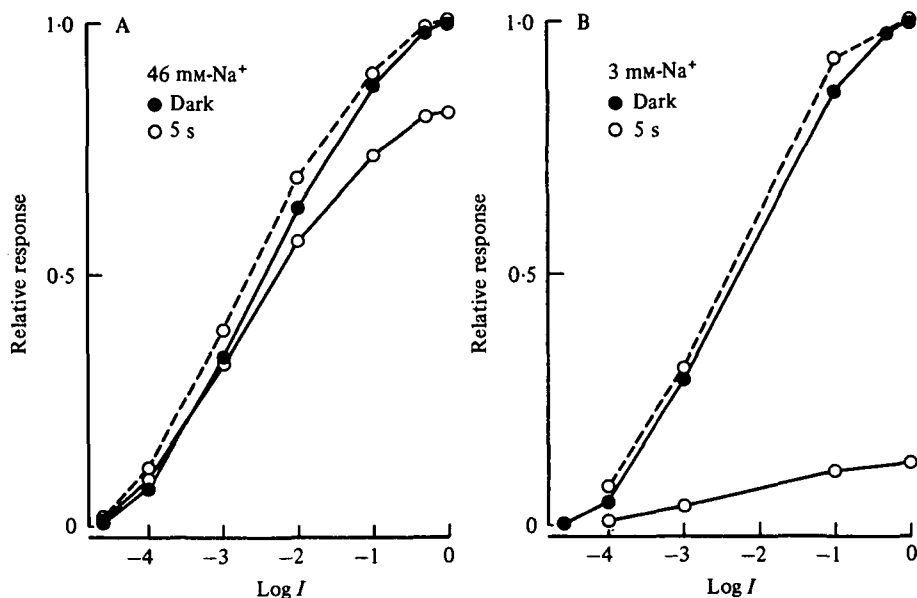
Fig. 3. Effects of reducing Na$^+$ from the perfusate on ERG intensity–response relations. The curves for the dark-adapted eye (closed circles) and for the eye 5 s after the end of a CD of 1 s duration (open circles) were obtained in 46 mM-Na$^+$ saline (A) and in 3 mM-Na$^+$ saline (B). In both A and B, solid lines were drawn relative to the saturated value for the dark-adapted eye. Dashed curves were normalized to the saturated value after the end of CD.
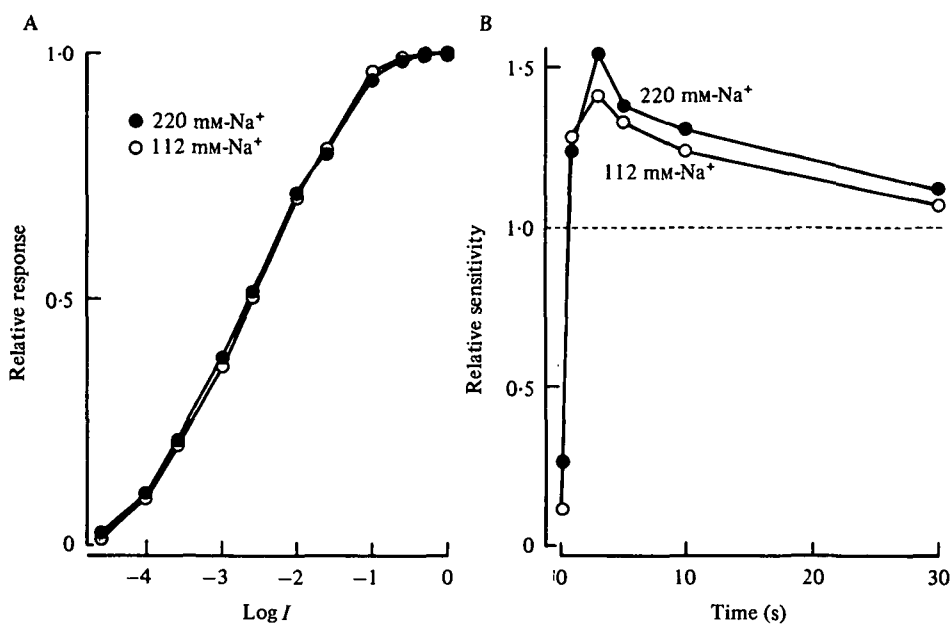

Fig. 4. Effects of reducing Na$^+$ from the perfusate on (A) normalized ERG intensity–response curves for the dark-adapted eye and (B) changes in sensitivities after a CD of 1 s duration obtained in normal saline containing 220 mM-Na$^+$ (closed circles) and in 112 mM-Na$^+$ saline (open circles).
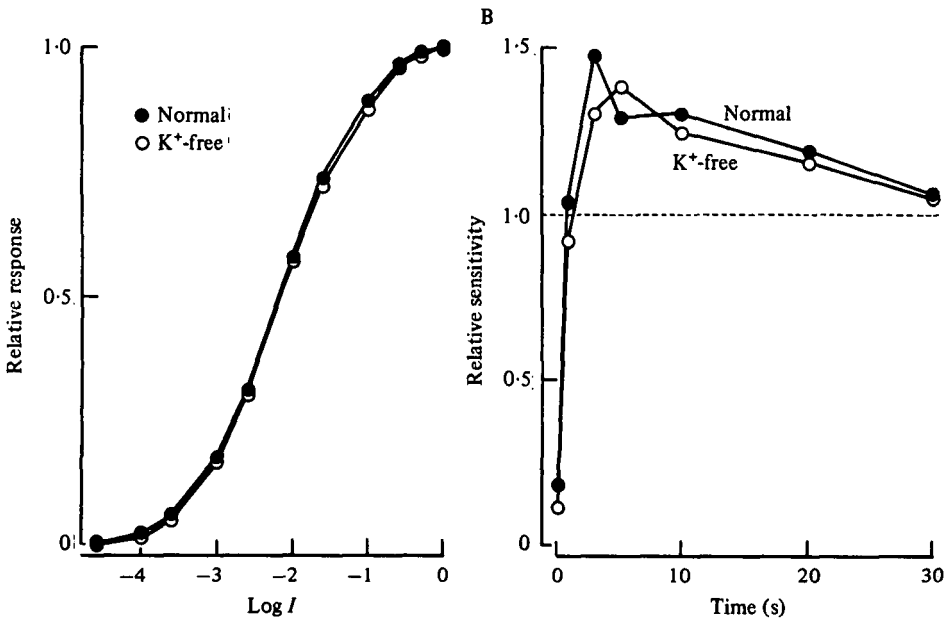
Fig. 5. Effects of reducing K+ from the perfusate on (A) normalized ERG intensity–response curves for the dark-adapted eye and (B) changes in sensitivities after a CD obtained in normal saline (closed circles) and in K+-free saline (open circles).

sensitivity reported above. The role of Na+ in regulating the saturated value of the ERG after the conditioning illumination was not investigated.

In K+-free saline, the dark-adapted intensity–response curve and the change in sensitivity after illumination were about the same as with those in normal saline (Fig. 5). Therefore, it is unlikely too that K+ plays a significant role in the hypersensitivity.

Effects of calcium ions

In contrast, Ca^{2+} had marked effects on the hypersensitivity. Fig. 6 shows the effects of changes in external Ca^{2+} concentration on ERG light responses to control light (C) presented to the completely dark-adapted eye, and test light (T) presented 5 s after the onset of a conditioning stimulus (T_1) and 5 s after the cessation of the conditioning stimulus (T_2). The amplitudes of the responses to C, T_1 and T_2 are given in Table 1.

Changes in external Ca^{2+} concentration had a small effect on light responses during the completely dark-adapted state, but a large effect during and after illumination. The intensity–response curves show that the sensitivity of the dark-adapted eye was somewhat greater in low-Ca^{2+} saline, and somewhat lower in high-Ca^{2+} saline (Fig. 7A). Hypersensitivity after illumination markedly increased to a maximum of about 2·0 in low-Ca^{2+} saline and markedly decreased to about 1·1 in high-Ca^{2+} saline (Fig. 7B). Maximum sensitivity was about 1·5 in normal saline. These results show that external Ca^{2+} plays an important role in determining the sensitivity of the eye especially during and after illumination.
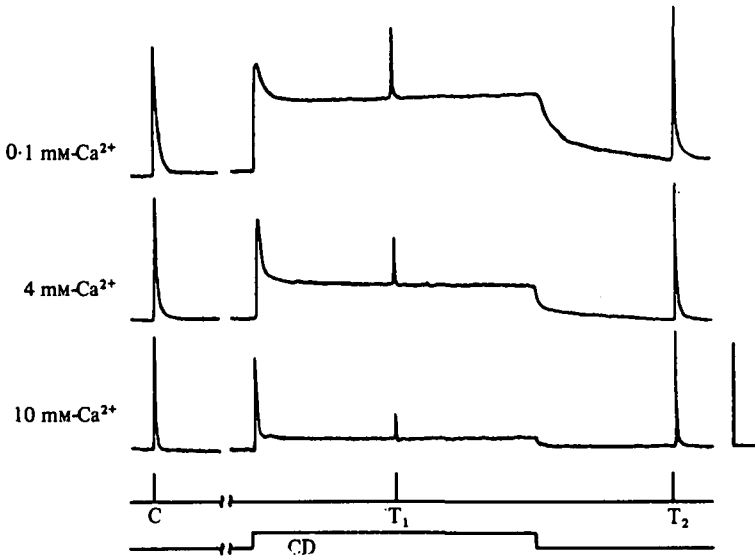
Fig. 6. Effects of changes in external Ca^{2+} concentration on ERG light responses obtained in 4 mM-Ca^{2+} (normal), 0·1 mM-Ca^{2+} and 10 mM-Ca^{2+} saline. C was given to the completely dark-adapted eye. T_1 was presented 5 s after the onset of a CD and T_2 5 s after the cessation of the CD. Calibrations: 1 mV, 1 s.

Table 1. *Effects of Ca^{2+} on ERG amplitude (mV)*

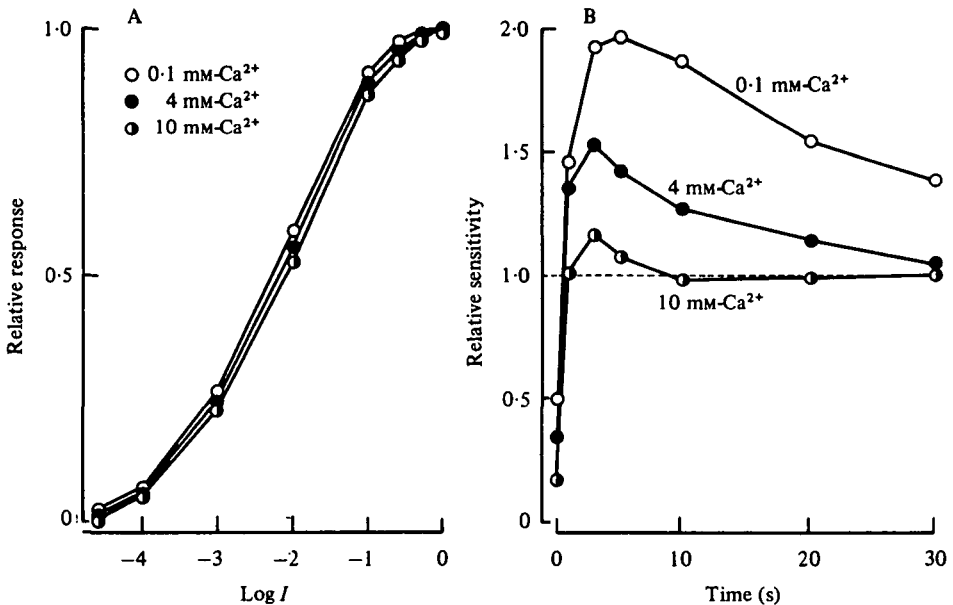| Perfusate/Stimulus	C	T_1	T_2
0·1 mM-Ca^{2+} saline	1·24	0·68	1·48
4 mM-Ca^{2+} saline	1·20	0·44	1·33
10 mM-Ca^{2+} saline	1·11	0·23	1·13

Fig. 7. Effects of Ca^{2+} concentration on (A) normalized ERG intensity–response curves for the dark-adapted eye and (B) changes in sensitivities after a CD obtained in 4 mM-Ca^{2+} (normal), 0·1 mM-Ca^{2+} and 10 mM-Ca^{2+} saline.
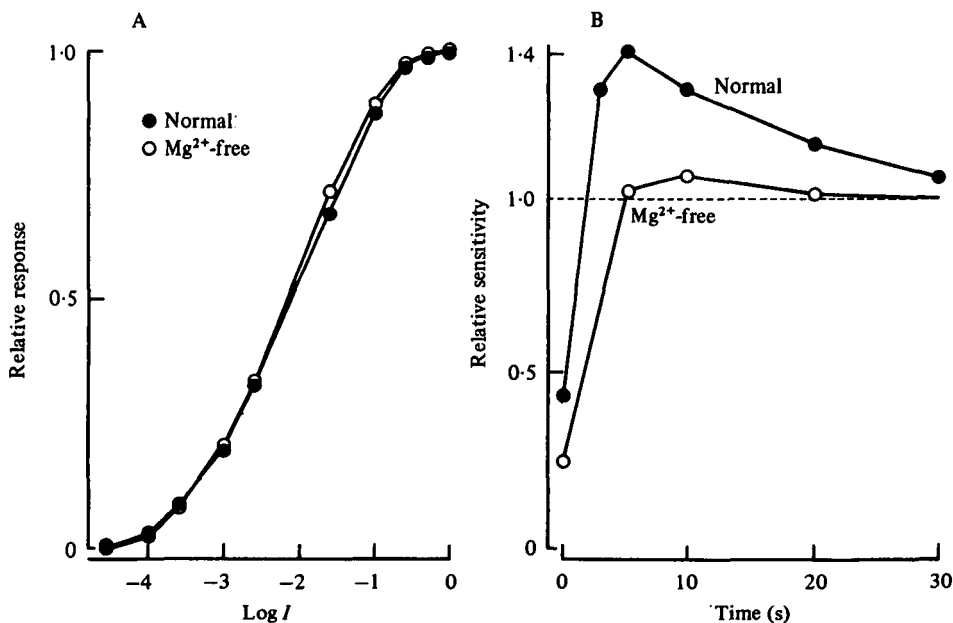
Fig. 8. Effects of reducing Mg^{2+} from the perfusate on (A) normalized ERG intensity–response curves for the dark-adapted eye and (B) changes in sensitivities after a CD obtained in normal saline (closed circles) and in Mg^{2+}-free saline (open circles).

Effects of magnesium ions

Intensity–response relations during the dark (Fig. 8 A) and the changes in sensitivities after illumination (Fig. 8 B) obtained with normal and Mg^{2+}-free saline show that Mg^{2+} had no effect in the dark-adapted eye. Mg^{2+}, however, had a marked effect on hypersensitivity. In normal saline, maximum sensitivity after illumination was about 1·4 but in Mg^{2+}-free saline, it was only about 1·05. These results show that Mg^{2+} is necessary for maintenance of hypersensitivity in the *Menemerus* eye.

DISCUSSION

It has been reported previously that Ca^{2+} plays important roles in controlling sensitivity of invertebrate photoreceptors. Lisman & Brown (1972) showed that during intracellular iontophoretic injection of Ca^{2+} into *Limulus* ventral photoreceptors, there was a progressive diminution of the light response. They postulated that light stimulation leads to an increase in intracellular Ca^{2+} which in turn reduces responsiveness to light stimulation. Direct measurement of intracellular Ca^{2+} concentration in *Limulus* ventral photoreceptors and *Balanus* photoreceptors indicates that illumination does induce an increase in the intracellular Ca^{2+} concentration (Brown & Blinks, 1974). Hanani & Hillman (1976) showed that the barnacle photoreceptor sensitivity either decreased or increased after exposure to illumination, and both phenomena were influenced by external Ca^{2+} concentration. In a study on the retina of the honey bee drone, Bader *et al.* (1976) showed that an increase in intracellular Ca^{2+} ■centration played a central role in light adaptation.

As shown in the present study, sensitivity of the spider anteromedian eye was als⬛ greatly affected by Ca^{2+}. When the external Ca^{2+} concentration is low, the sensitivity of the dark-adapted eye increases a little and hypersensitivity after illumination markedly increases. Yamashita & Tateda (1976) suggested that there were two antagonistic processes in photoreceptor cell of the AM eye. One process decreased the sensitivity during illumination (process I), the other process increased the sensitivity (process II), and a saturation of process II occurred more slowly than that of process I. The actual time course of light and dark adaptation corresponded to the summated time courses of processes I and II. They (1976, 1978) showed that respiration was necessary to maintain hypersensitivity, and process II appeared to be affected by respiratory activity suggesting that process II might be related to an active mechanism. The hypersensitivity phenomenon in spider eye may be explained by the following hypothesis. A light-dependent Ca^{2+}-pump which may be related to process II is present in the photoreceptor cells. Efflux of Ca^{2+} from the photoreceptor cells mediated by the light-dependent Ca^{2+}-pump is greater than influx of Ca^{2+} during illumination. The resulting decrease of intracellular Ca^{2+} concentration may continue for a short period after the cessation of illumination. As a result, the sensitivity of the photoreceptor cells following illumination may increase for a short period. When the external Ca^{2+} concentration is high, or Mg^{2+} concentration is low, influx of Ca^{2+} may be greater and/or the Ca^{2+}-pump may be less activated than in normal saline.

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