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Sensitivity Changes of Photoreceptor Cells of *Hirudo medicinalis* Caused by Changes in Extracellular Calcium Concentration*

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Abstract. Extracellular recordings from the vacoule of photoreceptor cells of *Hirudo medicinalis* L. were performed using microelectrodes. The cells were adapted by white light flashes given at constant intervals (20 s). Response height versus relative intensity curves obtained from the same cell in physiological saline (PS) and in bathing solutions of either a) lowered calcium contents (2 μM/l or less) or b) raised calcium contents (15 mM/l) were compared. The cells' adaptation state in PS was operationally defined by the ratio $Q = h_A/h_S$ where h_A is the response height evoked by the adapting flashes, and h_S is the corresponding saturation response height. Sensitivity changes were measured by the half saturation intensity shift. Lowering extracellular calcium resulted in:

1. The response height increased and the shape of the response became more rounded and prolonged.
2. The total resistance between the vacuole and outside decreased from 8.2 ± 1.4 MΩ ($n = 6$) in PS to 4.6 ± 0.4 MΩ ($n = 5$). The resistance was independent of the cells' adaptation state.
3. A change of the cells' sensitivity occured either in direction to light adaptation or in direction to dark adaptation. It depended functionally on the ratio Q:

a) if Q was less or equal to about 0.6 the cells' sensitivity increased. b) if Q was greater than 0.6 the cells' sensitivity diminished.

Raising extracellular calcium decreased the sensitivity of all cells tested independent of their adaptation states in PS. The results can be interpreted under the assumptions that 1. the sensitivity of leech photoreceptor cells is inversely proportional to the intracellular free calcium concentration and 2. intracellular calcium can interact with extracellular calcium in relatively dark adapted cells whereas in relatively light adapted cells the raise of intracellular free calcium is mainly effected by a release from intracellular

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stores. It is assumed that a Q value of about 0.6 separates relatively light adapted cells from relatively dark adapted cells.

Key words: Leech photoreceptors – Extracellular calcium – Excitation – Adaptation

Introduction

Intracellular recordings in invertebrate photoreceptors yield graded depolarizations after a light stimulus; the response being dependent on the instantaneous light stimulus as well as on the cells' adaptation state. The phenomenon of adaptation could be characterized as the cells' capacity to transiently reduce its excitability after a light stimulus. It is widely unknown to which molecular mechanisms the adaptation processes are originally related. It appears that light adaptation is correlated to an increase of the free intracellular calcium concentration (Ca_i^{2+}) in *Limulus* ventral photoreceptors (Brown and Blinks 1974; Brown and Lisman 1975; Brown et al. 1977; Stieve 1977; Fein and Charlton 1977; Maaz and Stieve 1980). This concept was qualitatively confirmed also in the honeybee drone (Bader et al. 1976) and in the squid (Pinto and Brown 1977).

The influence of extracellular calcium (Ca_o^{2+}) on light adaptation is, however, not uniform. Removal of Ca_o^{2+} diminished the light induced Ca_i^{2+} increase to undetectable amounts in photoreceptors of Balanus, but in the ventral eye of Limulus corresponding experiments led only to a small reduction of the light induced Ca_i^{2+} increase (Brown and Blinks 1974). This contrasts with Maaz and Stieve (1980) who measured the light induced absorption change of the Ca^{2+}-indicator arsenazo III, injected into Limulus ventral photoreceptors. After removing Ca_o^{2+} and waiting longer than Brown and Blinks (1974) the absorption change was undetectable. In another object, the retina of the cephalopod *Sepiola atlantica,* a decrease of Ca_o^{2+} does not lead to any change of sensitivity (Lipton et al. 1977).

Besides this the action of Ca_o^{2+} appears to depend on the adaptation state for the ventral eye of Limulus. Data from Lisman (1976), Martinez and Srebro (1976) and O'Day and Lisman (1979) seem to show, that Ca_i^{2+} is dependent on Ca_o^{2+} in dark adapted cells whereas in light adapted cells Ca_i^{2+} is released from intracellular stores and Ca_0^{2+} only partly contributes to light adaptation. The existence of Ca^{2+} storing compartments was conjectured by Brown and Blinks (1974) for Limulus ventral photoreceptors and was revealed by Perrelet and Bader (1978) for the honeybee, by Walz (1979) for *Hirudo medicinalis* and by Schröder et al. (1980) for the crayfish retina. These results seem to contradict the data from Stieve and Bruns (1980) who found a sensitization after removal of extracellular Ca_o^{2+} for light adapted ventral nerve photoreceptors of Limulus and no influence on the dark adapted state.

The purpose of this paper was to investigate the following problems. Among the different species the influence of Ca_o^{2+} on the sensitivity of photoreceptors is not uniform. In view of the somewhat conflicting results from other species a comparison with leech photoreceptor cells might be valuable. For leech

photoreceptor cells it is actually not known to what extent Ca_o^{2+} might change the sensitivity. And, if Ca_o^{2+} does influence the sensitivity of leech visual cells, it is asked whether this influence might depend on the degree of light or dark adaptation. In this paper evidence is reported for assuming a Ca^{2+} interaction between Ca_i^{2+} and Ca_o^{2+} only for relatively dark adapted cells.

Material

Isolated eye cups of *Hirudo medicinalis* L. were used in this study. The specimens were obtained from Hungary and kept at 16° C in aerated aquaria, which were illuminated by fluorescent light in a 12 h light, 12 h dark cycle.

Solutions

The preparation of the eye cup and the control experiments were performed in *Hirudo* physiological saline (referred to as PS) of the following composition (Sargent et al. 1977), mM/l: NaCl 115, KCl 4, $CaCl_2$ 7, Tris(hydroxymethyl)aminomethan 10, maleic acid 10, glucose 10.3, adjusted to pH 7.4 by 1 N NaOH. Low Ca^{2+} saline were prepared either by omitting Ca_o^{2+} or by adding 1 mM/l EGTA (Ethyleneglycol-bis-(β-amino-ethyl ether) N,N′-tetraacetic acid) to this saline. In the first case the remaining total Ca_o^{2+} content was 2 μM/l as measured by atomic absorption, in the second case approximately 1 nM/l as calculated from data from Portzehl et al. (1964). The chamber volume was 4 ml. During the experiment the PS could be continuously exchanged by syringes (50 ml) at a rate of 0.03 ml/s. A second pair of syringes was used for changing the solutions back to PS. Some experiments were performed in elevated Ca_o^{2+} saline by the addition of a small quantity of concentrated $CaCl_2$ under continuous stirring, resulting in a concentration of 15 mM/l $CaCl_2$.

Stimulation and Adaptation Procedure

The light of a tungsten filament source of 100 W (Leitz, Wetzlar, FRG) was passed through a condensor, an electronic shutter (Leitz), a heat absorbing filter (KG1, Schott, Mainz, FRG), neutral density filters (Schott) and a light pipe (Ø 5 mm). The unattenuated white light (log relative intensity of 0) at the site of the cell had an intensity of 17.5 mW/cm^2, corresponding to approximately $5 \cdot 10^{15}$ photons $\cdot$ cm$^{-2} \cdot$ s^{-1} at 510 nm, measured by a calibrated photometer (model 11A, PIN 10DF photodiode, UDT, Santa Monica CA, USA).

The photoreceptor cells were not adapted by continuous background lights, but by a dynamical method, using constant flash intensities I_A at constant stimulus intervals. The dependence of the light response on successively increasing stimulus intervals was tested (Fig. 1). Typically the cells did not become totally adapted to darkness with stimulus intervals of up to approximately 3 min. A constant interval of 20 s was taken as a compromize between

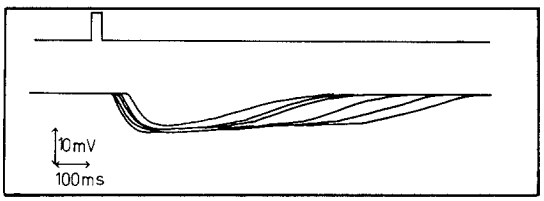

Fig. 1. Vacuole responses to constant bright intensity flashes (log relative intensity 0, corresponding to $5 \cdot 10^{15}$ photons $\cdot$ cm$^{-2} \cdot$ s^{-1} at 510 nm, 40 ms duration) in dependence of successively increasing stimulus intervals. The responses correlate from right to left with 120, 60, 30, 20, 10, and 5 s stimulus intervals. Intervals of more than 120 s often caused loss of the cell or smaller response heights

1. a moderate stimulation exposure within the lifetime of the cell ($\approx$ 1 h). 2. a fast collection and calculation of data and 3. the neccessity of adapting the cells to various adaptation states. With these limitations the cells were never totally dark adapted nor could they have been completely light adapted.

In the following the method for measuring the response height versus stimulus intensity curve is represented. The constant stimulus parameters (flash intensity I_A, duration 40 ms, interval 20 s) will cause the cells' adaptation state to change periodically. Test flashes of different intensities I_T given at the moment when a flash I_A was normally applied evoked responses which are characteristic both for I_T and I_A. Single test flashes were followed by several I_A flashes to restore the original adaptation state observed on a scope by responses of equal shape.

The response intensity curve could be fitted according to the equation $h_{\max} = h_S/(1 + [\sigma/\mathrm{I}])$, described by Naka and Rushton (1966). $h_{\max}$, h_S are the response heights at the test flash intensities I_T and at saturation, respectively, σ is the light intensity which resulted in the half saturation response height. Before the experiment was started the flash intensity I_A was varied from threshold through saturation and an appropriate fixed intensity I_A was chosen. The adapting flash intensity I_A, kept constant in one experiment, evoked response heights h_A, which ranged from some percent of h_S to saturation in distinct experiments. The more h_A reached the saturation value of h_S, the more the cell is considered to be light adapted. Conversely if h_A was only some percent of h_S the cell is assumed to be relatively dark adapted. In this paper the state of adaptation was operationally quantified by the ratio Q of h_A relative to h_S: $Q = h_A/h_S$. If the ratio was greater than 0.6, the cell was termed relatively light adapted and, for Q less or equal to 0.6, the cell was termed relatively dark adapted.

Sensitivity changes due to the change of the extracellular medium are operationally defined by $\Delta\sigma = \log\sigma - \log\sigma_{PS}$, disregarding the changes in steepness and saturation of the response intensity curves.

Recording

All experiments were performed at 17° C. Glass microelectrodes filled with 3 M/l KCl and with resistances between 20 and 80 MΩ were inserted into the

cells. As a reference, an Ag-AgCl electrode in 3 M/l KCl-Agar was used. The signal was amplified in a high impedance preamplifier (Model L/M-1, List, Darmstadt, FRG), displayed on a scope (Tektronix 5113) and fed into a PDP 11/34 computer. After digitizing with 1 kHz the response signal was displayed on the terminal screen and available for a hard-copy. The response was analysed by the computer for the latency t_l, time to peak t_p, decay time from peak to 50% of the maximal amplitude t_{50} and the maximal amplitude h_{max}. The latency was taken at the moment when the signal reached 5% of the maximal amplitude. Current injection pulses of 0.1 nA and 54 ms duration from a pulse generator (Model 302-T, WPI, Hamden, CT, USA) were used to determine the resistance of the cell in the dark and during the response. At the end of the experiment each characteristic parameter was plotted separately as a function of processing time. Comparing extra- and intracellular recordings from the vacuole and cytoplasm, respectively, it was found, that the time courses of the responses are obviously similar. This is confirmed by results from Fioravanti and Fuortes (1972) and was also stated by Fein and Charlton (1975) in Limulus ventral photoreceptors, by Stieve et al. (1978) in the crayfish retina and by Duncan and Pynsent (1977) at low intensities in the retina of *Sepiola atlantica*. The chosen time parameters of the response such as latency t_l, time to peak t_p and the half decay time t_{50} in particular did not differ by more than 10%. This justified recordings from the vacuole.

Results

1. Change of the Resistance R_{VE} between the Vacuole and Outside Caused by Removal of Ca_o^{2+}

As a morphological speciality of leech visual cells the membrane of all microvilli separates the cytoplasm from a compartment, called phaosome or vacuole. The vacuole which is surrounded by the cell communicates via clefts with the intercellular space (Lasansky and Fuortes 1969; White and Walther 1969; Walther 1970; Fioravant and Fuortes 1972).

Figure 2 shows the hypothetical equivalence circuit of a leech visual cell and responses recorded from he cytoplasm and from the vacuole in low Ca^{2+} saline. It was found that within experimental errors (15%) R_E was constant during the light response (Fig. 2a). On the contrary recordings from the cytoplasm (Fig. 2c) yield small resistances at the peak of the response and increasing resistances upon repolarization. A constant resistance R_{VE} during the light response was measured in PS as well as in low Ca^{2+} saline, but the value of R_{VE} was reduced from 8.2 ± 1.4 MΩ ($n = 6$) in PS by about 44% to 4.6 ± 0.4 MΩ ($n = 5$) in low Ca^{2+} saline. A change of the adaptation state left R_{VE} unchanged.

Recordings in the cytoplasm led to resistances between 26 and 40 MΩ in the dark in PS, that is the resistances were greater than R_{VE} by a factor of 3–5.

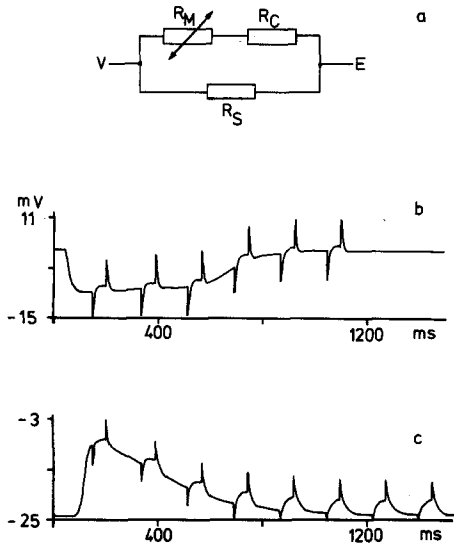

Fig. 2a–c. a Hypothetical equivalence circuit of the resistances between the vacuole V and the extracellular medium E. R_S, R_C, R_M are the resistances of the clefts, from E to the cytoplasm and from there across the microvilli to V, respectively. R_M is regarded as light sensitive. **b** and **c**: responses from the vacuole **b** and from the cytoplasm **c** for two cells in low Ca^{2+} saline (2 μM/l) superimposed with 54 ms pulses. In the vacuole the voltage drop to current pulses (0.1 nA) is almost constant, whereas in the cytoplasm the resistance is time dependent. The light stimulus of 40 ms duration started at zero time

2. Change of Response Parameters and Sensitivity After Lowering the Ca^{2+} Content of the Bathing Solution

a) at Constant Adaptation Flash Intensity I_A Throughout the Experiment. Penetrations through the cells' cytoplasm into the vacuole resulted in resting potentials which ranged from about +3 mV to −3 mV. Light stimuli caused hyperpolarizing responses (Fig. 1). After removal of Ca_o^{2+} the resting potential decreased by about 2.2 ± 1 mV ($n = 36$), but slowly returned to the control (after about 15 min).

In the following results of experiments with low Ca^{2+} saline and with low Ca^{2+} saline containing EGTA are represented simultaneously because no significant differences between the action of both salines were found. Before the PS was exchanged a response intensity curve and the corresponding value of Q was determined. The response heights h_{max} were fitted by the computer according to $h_{max} = h_S/(1+ (\sigma/I)^n)$. The exponent n in PS was $n = 1.28 \pm 0.27$ for 36 experiments. n did not change significantly when the flash intensity I_A was varied. The exchange of the saline to low Ca_o^{2+} had a dramatic effect on all parameters of the responses elicited by constant flashes I_A (Fig. 3). While the latency increased with a delay, t_p, t_{50} and h_A rose with an initial step. The shape of the response became prolonged and more rounded (Fig. 4). While the PS was changed to low Ca^{2+} saline the response height h_A rose, became constant for at least 10 min and then slowly decreased. If the cell remained for approximately 10 min longer in low Ca^{2+} saline it became insensitive to light and the response could not be raised again by Ca^{2+} addition. In the stationary phase and also in the falling phase of h_A, however, the effect was almost reversible and the original response parameters could be restored (Fig. 3). On the other hand a reduction of I_T or I_A in low Ca^{2+} saline failed to simultaneously restore all parameters to the values in PS.

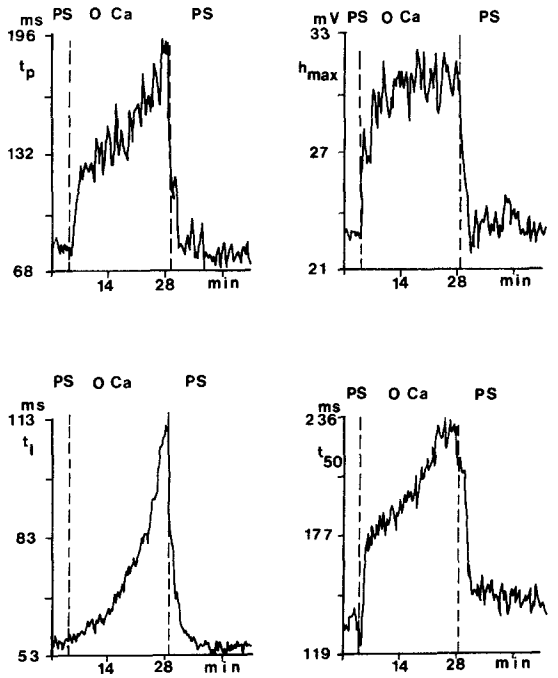

Fig. 3. Latency t_l, time to peak t_p, half decay time t_{50} and response height h_{max} as characteristic parameters of the response versus processing time. The dashed lines indicate the beginning of the change from PS to low Ca^{2+} saline (2 µM/l) and back to PS. The cell was relatively dark adapted (Q = 0.5, log relative intensity of the constant adaptation flash intensity I_A: −2.3. Stimulus interval 20 s, stimulus duration 40 ms, 17° C

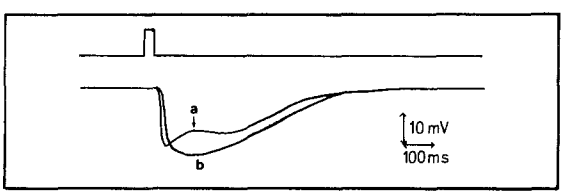

Fig. 4a and b. Vacuole responses to bright flashes in PS **a** and after the change to low Ca^{2+} saline **b** (Ca_o^{2+} = 2 µM/l). The white light stimulus (40 ms duration, 20 s interval, log relative intensity −1) is shown in the upper trace. The dip in **a** disappeared in low Ca^{2+} saline **b**

In the stationary phase of h_A the response intensity curve was measured in low Ca^{2+} saline. Figure 5 compares the response intensity curves in PS and in low Ca^{2+} saline for two cells, one of which was relatively dark adapted ($Q \leq 0.6$), the other relatively light adapted ($Q > 0.6$). Interestingly in most experiments the two response intensity curves in PS and in low Ca^{2+} saline intersected each other. The characteristic parameters of the response intensity curve, the exponent n and the saturation amplitude h_S increased slightly (see Table 1). In PS as well as in low Ca^{2+} saline the response intensity curves cover about 1.5 log units of intensity I_T from 10% to 90% of saturation. In this range of intensity the times t_p and t_l decreased whereas t_{50} increased semilogarithmically (Fig. 6). The increase of h_A in time seemed to be independent of Q.

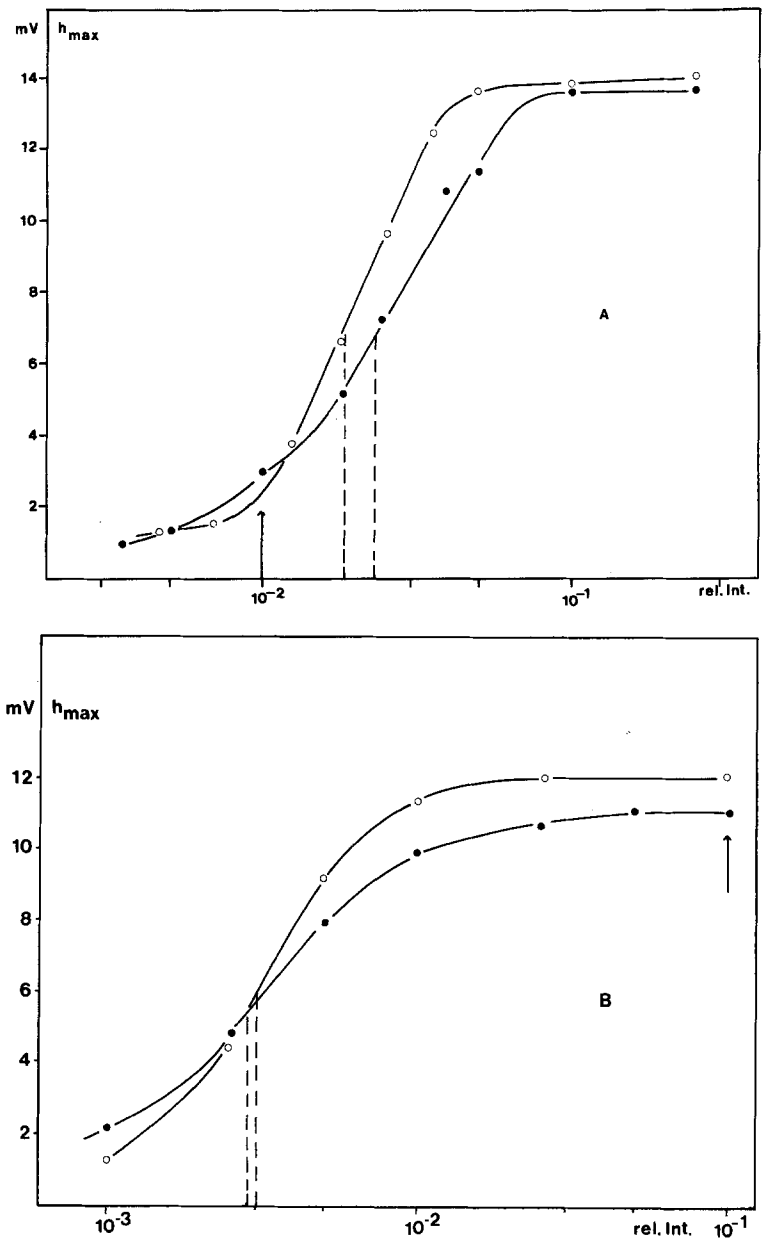

Fig. 5 A and B. Response height versus intensity curves in PS and in low Ca^{2+} saline (2 μM/l) for two cells which were relatively dark adapted **A** and relatively light adapted **B**. The adapting flash intensity (stimulus duration 40 ms, interval 20 s) is marked by the arrow. ● PS, ○ Ca^{2+} deficient saline (Ca^{2+} = 2 μM/l). The dashed lines indicate the half saturation intensities

Table 1. Response parameters (h_A, t_{50}) and characteristic parameters of the response intensity curves (h_S, n, $\Delta\sigma$) in PS and after the change to a) low Ca^{2+} saline (0 Ca, $c_{Ca} \approx 2$ µM/l) b) low Ca^{2+} saline, containing EGTA (0 Ca + EGTA, $c_{Ca} \approx 1$ nM/l) c) raised Ca^{2+} saline (+Ca, $c_{Ca} = 15$ mM/l). The PS contained 7 mM/l Ca^{2+}. In these experiments the adapting flash intensity I_A was kept constant throughout the experiment (duration 40 ms, interval 20 s). In some experiments I_A was reduced (d) after the change to low Ca^{2+} saline to maintain the value of h_A in PS (termed 0 Ca, red. I_A). t_{50} and h_A were taken shortly before the saline was changed and in the stationary phase after the change (see Fig. 3). h_S, n, $\Delta\sigma$ are the saturating response height, the exponent in the Naka-Rushton equation and the shift of the response intensity curve, respectively. The table is subdivided into two groups of experiments with relatively dark adapted cells ($Q = h_A/h_S \leq 0.6$) and with relatively light adapted cells ($Q > 0.6$). Mean and standard deviation, the number of experiments in brackets

		a PS ⟶ 0 Ca	b PS ⟶ 0 Ca + EGTA	c PS ⟶ + Ca	d PS ⟶ 0 Ca, red.
$Q \leq 0.6$	h_A [mV]	7.8 ± 3.5 → 10.2 ± 1 (8)	6 ± 0.9 → 7.8 ± 0.6 (3)	4.4 ± 1.2 → 3.2 ± 0.4 (2)	3 ± 1.4 → 3 ± 1.4 (3)
	t_{50} [ms]	129 ± 34 → 157 ± 15 (8)	134 ± 40 → 159 ± 14 (3)	192 ± 43 → 199 ± 6 (2)	184 ± 41 → 263 ± 39 (3)
	h_S [mV]	22.6 ± 6.1 → 24.6 ± 5.2 (8)	15.7 ± 5.8 → 16.6 ± 5.3 (3)	11.9 ± 2.1 → 6.6 ± 1.5 (2)	9.1 ± 3.6 → 9.1 ± 3.6 (3)
	n	1.31 ± 0.26 → 1.36 ± 0.3 (8)	1.12 ± 0.05 → 1.26 ± 0.2 (3)	0.99 ± 0.4 → 0.91 ± 0.05 (2)	1.1 ± 0.15 → 1.1 ± 0.15 (3)
	$\Delta\sigma$	− 0.13 ± 0.03 (8)	− 0.07 ± 0.03 (3)	0.2 ± 0.1 (2)	− 0.32 ± 0.02 (3)
$Q > 0.6$	h_A [mV]	9.4 ± 1.9 → 12.8 ± 1.1 (14)	13 ± 3.1 → 15 ± 0.9 (8)	9.1 ± 2 → 5.7 ± 0.5 (4)	11.9 ± 2.6 → 11.9 ± 2.6 (5)
	t_{50} [ms]	236 ± 52 → 278 ± 18 (14)	256 ± 73 → 294 ± 14 (8)	241 ± 92 → 262 ± 10 (4)	189 ± 61 → 232 ± 9 (5)
	h_S [mV]	14.1 ± 5.3 → 17.8 ± 6 (14)	13.8 ± 4.8 → 16.5 ± 5.1 (8)	10.6 ± 2 → 5.5 ± 1.7 (4)	11 ± 3.3 → 11 ± 3.3 (5)
	n	1.4 ± 0.4 → 1.5 ± 0.4 (14)	1.28 ± 0.24 → 1.36 ± 0.3 (8)	1.36 ± 0.29 → 1.37 ± 0.25 (4)	1 ± 0.1 → 1 ± 0.2 (5)
	$\Delta\sigma$	0.07 ± 0.06 (14)	0.08 ± 0.07 (8)	0.1 ± 0.1 (4)	0.01 ± 0.1 (5)

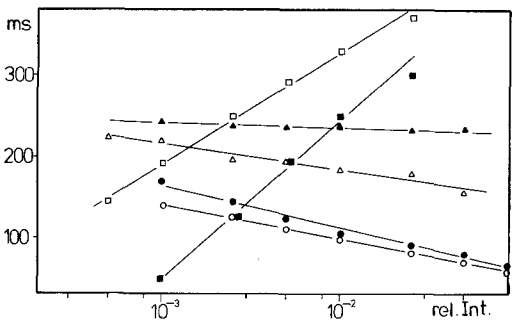

Fig. 6. Characteristic time parameters of the response in PS against relative test flash intensity I_T. Each parameter was measured at two adaptation states, characterized by $Q = h_A/h_S$ (see Methods)

Q	t_l	t_p	t_{50}
0.5	○	△	□
0.97	●	▲	■

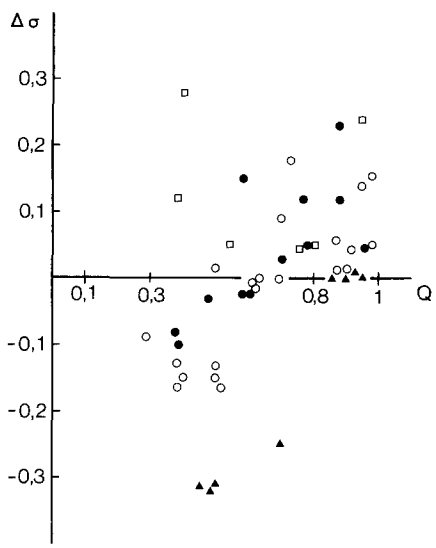

Fig. 7. Half saturation intensity shifts, $\Delta\sigma$, due to altered Ca_o^{2+}, versus the adaptation state determined in PS and operationally expressed by $Q = h_A/h_S$. Each point represents one cell. The change of the bathing solution resulted in $\Delta\sigma = \log(\sigma/\sigma_{PS})$. ○: change to low Ca^{2+} saline (2 µM/l), ●: change to EGTA-Ca^{2+}-free saline ($Ca_o^{2+} \approx 1$ nM/l). □: raised Ca^{2+} saline (15 mM/l), ▲: change to low Ca^{2+} saline at reduced I_A to keep h_A constant at the value in PS

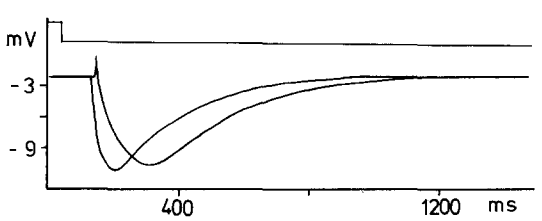

Fig. 8. Responses at different flash intensities I_A and Ca_o^{2+} concentrations in the salines. Left response: PS. Right response: in low Ca^{2+} saline (2µM/l) at reduced flash intensity I_A in order to maintain the value of h_A in PS. Stimulus duration 40 ms, interval 20 s. The spike after the beginning of the response was also present in PS but was hidden in the fast negative slope

In PS at bright light flashes (I_A or I_T) the response showed a positively directed trough shortly after the first peak of the response. This trough was never observed in low Ca^{2+} saline (Fig. 4). Figure 7 demonstrates that the shift $\Delta\sigma$ of the response intensity curves could be correlated to the value of Q in PS. If h_A was less than 60% of the corresponding value of h_S, ($Q \leqslant 0.6$) the shift $\Delta\sigma$ due to Ca_0^{2+} removal was found to be negative. In contrast, $\Delta\sigma$ was positive in experiments if h_A was greater than 60% of h_S.

b) at Constant h_A (I_A Reduced). The adapting flash intensity I_A was reduced during the change to low Ca^{2+} saline in order to keep the response height h_A constant. These experiments should show a) how a changed flash intensity I_A could influence the effect of low Ca^{2+} saline and b) how low Ca^{2+} saline changes the time parameters at constant h_A. The reduction of h_A causes the cell to sensitize. It was found that the shifts $\Delta\sigma$ were more negative than for the experiments with constant I_A (Fig. 7). The exponent n and the saturation amplitude h_S did not change. The time parameter t_{50} was prolonged (Fig. 8) to a greater extent than for constant I_A (see Table 1).

3. Change of Response Parameters and Sensitivity After Raising the Ca^{2+} Content of the Bathing Solution at Constant Flash Intensity I_A Throughout the Experiment

The results presented above are now contrasted with experiments with raised Ca_0^{2+} (15 mM/l). The resting potential of the vacuole increased by about 1.8 ± 0.9 mV ($n = 5$) in raised Ca^{2+} saline, but slowly returned to the control.

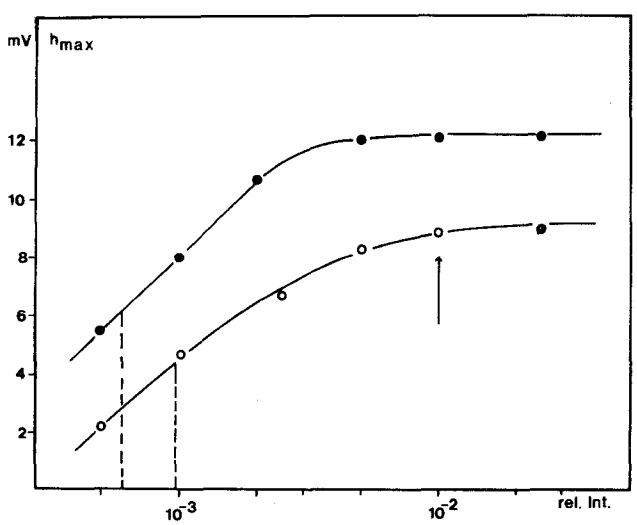

Fig. 9. Response height versus intensity curves in PS (●) and in saline with raised Ca_0^{2+} (○, 15 mM/l). Half saturation intensities are indicated by dashed lines, the adapting flash intensity by the arrow. The cell was relatively light adapted in PS ($Q = 0.95$). Stimulus interval 20 s, duration 40 ms

While the half decay time t_{50} increased only slightly, the response height h_A was reduced (see the Table 1). After changing the solution back to PS the original values of the response parameters could be restored. Regarding the response intensity curves it is conspicious that the shift $\Delta\sigma$ was positive for both $Q > 0.6$ and $Q \leqslant 0.6$ (Fig. 7). The exponent n slightly decreased with respect to PS, while the saturating response height h_S was strongly diminished. In contrast to the change to low Ca^{2+} saline it was found that the two response intensity curve do not intersect (Fig. 9). Obviously the effects of raised Ca^{2+} saline were not quite opposite to the effects of low Ca^{2+} saline in respect to the parameters of the response and to the response intensity curves.

Discussion

1. The Resistance R_{VE} and the Potential Between the Vacuole and Outside

For leech visual cells an equivalent circuit as shown in Fig. 2a is assumed. It consists of the resistance of the clefts R_S in parallel to the series of the resistance cytoplasm-vacuole R_M and the resistance cytoplasm-outside R_C. It has been hypothesized (Walther 1970; Fioravanti and Fuortes 1972) that the only resistance which is reduced upon illumination is R_M. The fact that R_{VE} was found to be constant during the light response indicates that R_S is small compared to R_C and/or R_M. R_{VE} is mainly determined by the light insensitive resistance R_S. This is further supported by the finding that cytoplasmatic recordings yielded resistances 3–5 times greater than R_{VE}. In low Ca^{2+} saline R_{VE} decreased but remained constant during the light response (Fig. 2b). At the same time the response height h_A increased. Both effects could be explained within the circuit diagram. It is assumed that all resistances are reduced, but R_S remains small compared to R_C and/or R_M. The light induced current which is presumably limited by R_C and/or R_M, increases after diminution of R_C and/or R_M. This could cause the raise of the response height as well as its decline after prolonged stay in low Ca^{2+} saline (Fig. 3).

It is not clear whether the potential drop measured after the cell had been impaled in PS arises from an electrode tip potential or whether it is of biological meaning. A potential of about zero mV could be explained – in the simplest approach – by equal mediums in the vacuole and outside. Reduction or rise of Ca_o^{2+} causes Ca^{2+} to diffuse out of or into the vacuole, respectively, leading to the slow potential changes (see Results). The slow return to the control value indicates a hindered diffusion across the clefts and/or a slow active process. It could be assumed that the functional meaning of the vacuole is causally related to the possibility of a better control and maintenance of the bathing solution near the microvilli. This would cancel out the disadvantage of a slightly reduced light induced current by R_S.

2. *Changes of Sensitivity due to the Change of Extracellular* Ca_o^{2+}

It is a wide accepted concept that the sensitivity of a photoreceptor cell can be described by the half saturation intensity σ, within a response intensity curve. Although the steepness and the saturation response height changes the cells' sensitivity, these parameters were disregarded for simplicity.

After changing the Ca_o^{2+} content of the saline the most striking observation with respect to the response intensity curves was that their shifts could be positive or negative. The ratio Q of 0.6 apparently separates negative from positive shifts after reduction of Ca_o^{2+}. The following discussion assumes that 1. relatively dark adapted cells can be characterized by $Q \leqslant 0.6$ and relatively light adapted cells by $Q > 0.6$. 2. Light adaptation is correlated to a transient increase of the free intracellular Ca^{2+} concentration. 3. Ca_i^{2+} interacts with Ca_o^{2+}, in an active or passive process, only for relatively dark adapted cells. The first assumption is derived from Fig. 7 and is of practical meaning. The second and third assumption is taken from other authors (see Introduction). Indirect evidence for the second hypothesis which might also be valid for the leech, is obtained from the fact that intracellular EGTA injection into leech visual cells resulted in a tremendous prolongation of the light response (unpublished). The condition $Q \leqslant 0.6$ was obtained in most experiments by adapting with flash intensities of less than 1% of the intensity used in the test of Fig. 1. If h_{max} approached saturation for a 20 s stimulus interval after a strong flash (Fig. 1), dimmer flashes would cause saturation of h_{max} more than ever.

1. I_A *= constant, low* Ca_o^{2+} *–* $Q \leqslant 0.6$. The negative shifts of the response intensity curves for relatively dark adapted cells (Figs. 5, 7) can be interpreted in two ways: either the concentration of Ca_i^{2+} is greater than Ca_o^{2+} or Ca_i^{2+} is actively diminished. The second alternative was favoured for Limulus ventral photoreceptors (Maaz and Stieve 1980). For leech visual cells Ca_i^{2+} concentrations are not yet known.

2. I_A *= constant, low* Ca_o^{2+} *–* $Q > 0.6$. The fact that in PS relatively light adapted cells reduce their sensitivities (Fig. 7) after Ca_o^{2+} removal supports the assumption that intracellular Ca_i^{2+} could be released probably from intracellular stores. A possible intracellular organelle in leech visual cells could be the endoplasmatic reticulum which was found to be able to accumulate Ca^{2+} under ATP consumption (Walz 1979). The mechanism of triggering a Ca^{2+} release after the reduction of Ca_o^{2+} remains unclear. It is assumed, however, that under this experimental condition ($Q > 0.6$, low Ca_o^{2+}) the release of Ca^{2+} from stores is increased with respect to PS ($\Delta\sigma > 0$) and/or the Ca^{2+} permeability is reduced from inside the cell, counteracting a loss of Ca_o^{2+}. This is consistent with Stieve (1977) who proposed that Ca^{2+} might act from both sides of the receptive membrane and might in/activate light channels.

3. I_A *= constant, high* Ca_o^{2+} *(15 mM/l) –* $Q \leqslant 0.6$. The concept of a Ca^{2+} communication for relatively dark adapted cells holds also for an interpretation

of these experiments. Ca_o^{2+} might enter the cell and thus desensitize it ($\Delta\sigma > 0$, Fig. 7).

4. I_A = constant, high Ca_o^{2+} (15 mM/l) − $Q > 0.6$. Ca^{2+} and Na^+ are suggested to compete for extracellular binding sites closing and opening light channels (Stieve 1977). High Ca_o^{2+} could therefore decrease the light induced inward current which is carried presumably by Na^+ (Millecchia and Mauro 1969). This effect may contribute, among other possible mechanisms, to a reduction of sensitivity ($\Delta\sigma > 0$, Fig. 7).

5. I_A reduced, low Ca_o^{2+}. In general a reduction of the adapting flashes I_A causes the cell to undergo dark adaptation to a certain degree. The shifts of the response intensity curves should then be more negative with respect to constant I_A, provided that the action of low Ca^{2+} saline at reduced I_A remains unchanged. The results presented in Fig. 7 confirm this conclusion. Even relatively light adapted cells showed an increase of sensitivity. A reduction of I_A improves the effectiveness of Ca_o^{2+} in sensitizing the cells and supports the idea of a Ca^{2+} interaction only for relatively dark adapted cells.

The dip in the response, found in PS could not be generated by bright test flashes in low Ca^{2+} saline. This finding is similar to data reported by Schmidt et al. (1978) for light adapted photoreceptors of Hermissenda crassicornis and by Hanani and Shaw (1977) for barnacle photoreceptors. One component of the dip was interpreted as a potassium conductance increase induced by a Ca^{2+} influx.

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