Electrogenic Na^+-Ca^{2+} Exchange Contributes to the Light Response of Fly Photoreceptors

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The response of fly photoreceptors to light stimuli of high intensity consists of a fast transient depolarization caused by the activation of light-dependent channels and a delayed afterdepolarization. The afterdepolarization was reversibly reduced or abolished when the extracellular Na^+ had been replaced by Li^+, tris or choline. Na^+ specificity, the dependence on the concentrations of Na^+ and Ca^{2+} as well as the polarity of the effect suggest that electrogenic Na^+-Ca^{2+} exchange contributes to the afterdepolarization. Prolonged incubation in Na^+-free media also led to the reversible suppression of the fast response component, which is most probably due to an increase in the intracellular Ca^{2+} concentration caused by the inhibition of Na^+-Ca^{2+} exchange. Evidence is presented that the Na^+-Ca^{2+} exchange system in fly photoreceptors also transports Sr^{2+}.

Introduction

The Na^+-Ca^{2+} exchange system is one of the major mechanisms involved in the control of the intracellular concentration of Ca^{2+} (Ca_i). This transport mechanism has been investigated in a variety of cellular systems, particularly in heart muscle cells, squid axon and vertebrate photoreceptors. The Na^+-Ca^{2+} exchange mechanism is a carrier-mediated transport system in which the transmembrane movement of Ca^{2+} is coupled to the movement of Na^+ in the opposite direction (for review see [1, 2]). The system normally functions as a mechanism for pumping Ca^{2+} out of the cell (forward mode), but is has also been shown that it can operate in the reverse direction (reverse mode). The Na^+-Ca^{2+} exchange is electrogenic, *i.e.*, it is associated with the transfer of net charge across the cell membrane, and there is good evidence that the stoichiometry of the transport is 3 Na^+: 1 Ca^{2+} [3]. However, recent studies indicate that the Na^+-Ca^{2+} exchange in vertebrate photoreceptors involves the transport of K^+, in that 4 Na^+ are transported for 1 Ca^{2+} and 1 K^+ [4, 5].

Several lines of evidence indicate that also in invertebrate photoreceptors Na^+-Ca^{2+} exchange participates in the regulation of Ca_i, which plays an important role in controlling the sensitivity of the receptor cells (see [6, 7]). Thus in *Limulus* ventral photoreceptor cells, manipulations which decreased the Na^+ gradient across the cell membrane induced an increase in Ca_i [8–11], which is probably due to a Na^+-Ca^{2+} exchange system that can operate in forward and reverse direction [12]. Furthermore, a light-induced Na^+-dependent accumulation of Ca^{2+} in the extracellular space of the bee retina has been found ([13–15], compare [16]).

In insects Na^+-Ca^{2+} exchange has been demonstrated for the first time by showing that a component of the photoreceptor response to light critically depends on the gradients of Na^+ and Ca^{2+} across the cell membrane [17, 18]. However, this "transient post-illumination afterpotential" (TA) was only observed when the cells were under hypoxia or treated with ruthenium red which inhibits active Ca^{2+} uptake by intracellular organelles. In this report it is shown that Na^+-Ca^{2+} exchange contributes to the light response of fly photoreceptors also under normal conditions. Furthermore, the significance of the Na^+-Ca^{2+} exchange system for the functioning of fly photoreceptors is demonstrated by showing that the receptor response is reversibly suppressed if Na^+-Ca^{2+} exchange is inhibited.

Materials and Methods

Intracellular responses of the photoreceptors R 1-6 were recorded from male white eyed blowflies (*Calliphora erythrocephala,* Meig., chalky mu-

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tant), reared on liver. The preparation of the fly and the experimental set-up were previously described [19]. In short, the fly was mounted in a holder and its head was horizontally hemisected close to the equator of the eye (cut preparation). The holder was fitted to a perfusion chamber in which the head was permanently superfused. In most experiments the cells were impaled in a saline which contained 130 mM NaCl, 10 mM $CaCl_2$ and 10 mM Hepes (N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid) adjusted to pH 7.0. In some experiments salines were used in which the Ca^{2+} concentration was reduced to 3 mM, 1 mM or 0.1 mM without osmotic compensation. In the experiments the initial superfusion medium was temporarily exchanged for a medium in which Na^+ was replaced by Li^+, tris (tris(hydroxymethyl)-aminomethane) or choline, or for a medium which contained 100 mM $MgCl_2$, $CaCl_2$, $SrCl_2$ or $BaCl_2$, buffered with 10 mM Hepes (pH 7.0). Since NaOH was used to adjust the pH, the Na^+ concentration of these "Na^+-free" salines was about 2 mM. The flow rate was 5 ml/min, so that the fluid in the perfusion chamber (0.5 ml) was renewed every 6 s. In the tissue the extracellular medium was completely exchanged within less than 30 s as estimated from the time course of the depolarization of the cells induced by increasing the extracellular K^+ concentration. The cells were impaled under microscopic control in dim red light which is not absorbed by the fly's visual pigment. The orientation of the preparation was such that the photoreceptors were illuminated perpendicular to their long axes. The photoreceptors were stimulated *via* the microscope objective by a white xenon flash (halfwidth 0.5 ms) filtered through a pair of cut-off filters to exclude UV and IR radiation. A single flash led to a maximum absorption of 10^8 light quanta which activate most of the cell's visual pigment (see [20]). The preparations were dark adapted for 3 min before the experiment. All recordings presented in this report were DC recordings. The experiments were carried out at room temperature.

Results

Like in most invertebrates, the photoreceptors of the fly depolarize transiently upon stimulation with a short light flash. At low stimulus intensities the time course of the photoreceptor response is rather simple: following a latency period of a few ms, the cell begins to depolarize and, after reaching a minimum in membrane potential, it steadily repolarizes to the resting potential. It is generally accepted that this response is due to an influx of Na^+ through light-activated ion channels followed by an efflux of K^+. At higher stimulus intensities the time course of the responses is more complicated as seen in Fig. 1a (left handside) which shows a typical response of a photoreceptor cell to an intense flash of light at an extracellular Ca^{2+} concentration of Ca_o = 10 mM. Two response components can be distinguished: an initial positive peak of large amplitude (62 mV) which is caused by the activation of the light-dependent ion channels and, after a transient maximum of the membrane potential, an afterdepolarization of minor amplitude (10 mV). The initial peak started 2 ms after the stimulus and reached its maximum about 10 ms later. The subsequent afterdepolarization became maximal 150 ms after the flash and lasted about 20 s.

The afterdepolarization was completely suppressed shortly after the Na^+ in the superfusion medium had been replaced by Li^+, whereas the peak component remained essentially unaffected (Fig. 1a, middle traces). Merely a small reduction in peak amplitude by less than 10% and a slight acceleration of the repolarizing phase were observed. The effects were reversible: in particular, the afterdepolarization always reappeared after restoration of the initial conditions (Fig. 1a, right handside), even in experiments in which the regeneration of the peak response was absent or incomplete. The same results were obtained when Na^+ was replaced by choline or tris (Fig. 1b), except that the amplitude of the peak component was more reduced than in case of Li^+. These results indicate that the suppression of the afterdepolarization is due to the removal of Na^+ but not to the presence of the particular ion species by which Na^+ is replaced.

An afterdepolarization was detected only at high stimulus intensities: the absorption of 10^4 quanta was necessary to evoke an amplitude of about 1 mV. For comparison, a peak response of the same amplitude is elicited by the absorption of 50 quanta; when 10^4 quanta were absorbed the amplitude of the peak response ranged from 25 to 30 mV (Fig. 2b). At stimulus intensities of up to

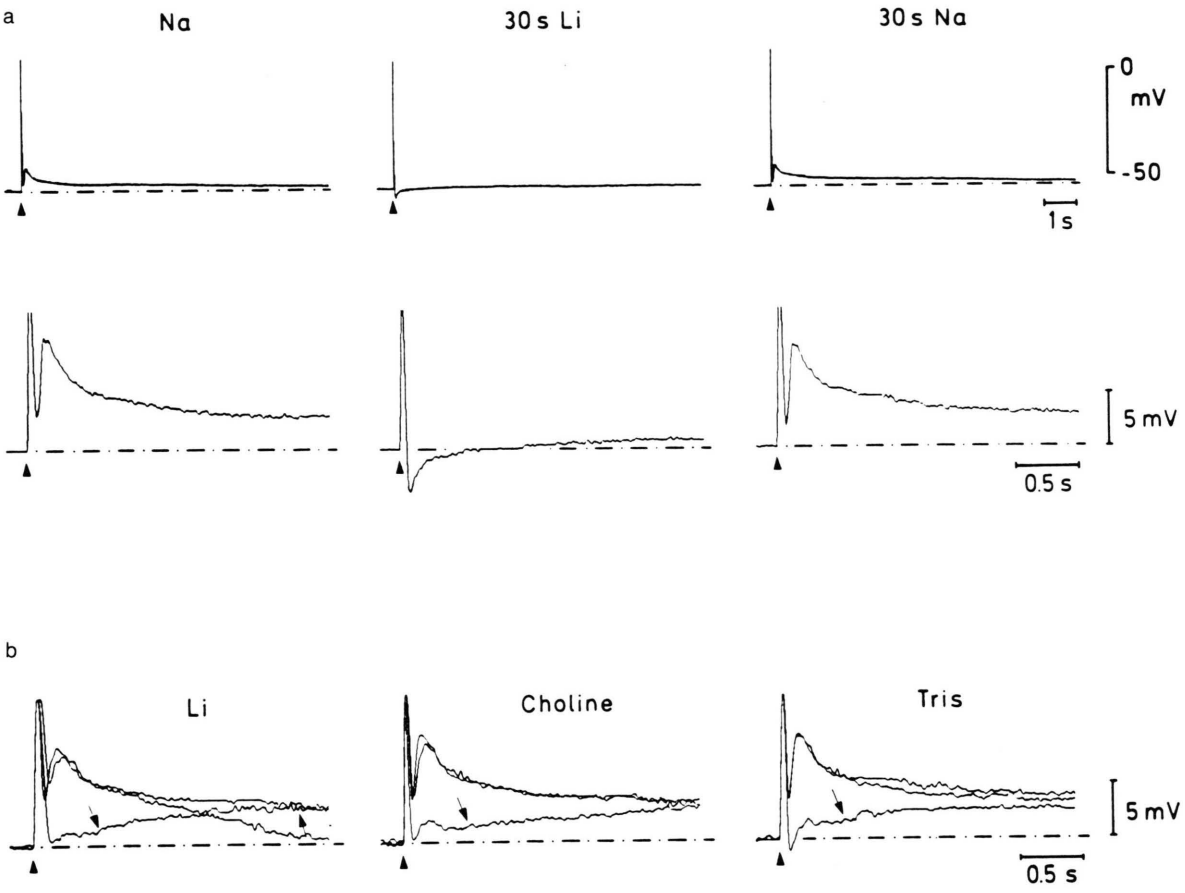

Fig. 1. Reversible suppression of the afterdepolarization by replacing extracellular Na^+ by other monovalent cations (Ca_o = 10 mM). Light flashes (arrowheads) led to 3×10^7 photon absorptions. a) Responses of a photoreceptor cell before (Na) and 30 s after Na^+ had been replaced by Li^+ (30 s Li) and finally 30 s after Li^+ had been exchanged again for Na^+ (30 s Na). The lower set of traces shows the respective responses at a magnified scaling (peak response out of scaling). Note that replacing Na^+ by Li^+ left the initial peak response almost unaffected but essentially suppressed the afterdepolarization. b) Superimposed responses of a photoreceptor cell recorded before, 60 s after replacing Na^+ by Li^+, choline or tris, and 60 s after restoration of the initial conditions (peak response out of scaling). In each set the upper 2 traces represent the responses derived in the presence of Na^+ before and after the exchange; responses in Li^+, choline or tris are marked by arrows. Mean resting potential in the Na^+ medium: −47 mV; Li^+: −48 mV; choline/tris: −52 mV.

10^7 quanta absorbed the afterdepolarization consisted of a fast depolarization followed by a much slower repolarization (Fig. 2a). At higher intensities the decay of the afterdepolarization proceeded in two distinct phases.

In order to suppress the afterdepolarization completely, most of the extracellular Na^+ had to be removed. Fig. 3 demonstrates that an afterdepolarization was still evident at an extracellular Na^+ concentration of Na_o = 35 mM. The difference between the response traces recorded at Na_o = 18 and 2 mM is probably not significant since a regeneration of the afterdepolarization was not observed when Na_o was increased again from 2 to 18 mM. However, a regeneration occurred when Na_o was raised to 35 mM (not shown).

The significance of extracellular Na^+ for the generation of the afterdepolarization was clearly evident at Ca_o = 10 mM (Fig. 1, 3 and 4). At lower Ca^{2+} concentrations larger afterdepolarizations were observed which, after replacement of Na^+, were not completely suppressed. But at Ca_o =

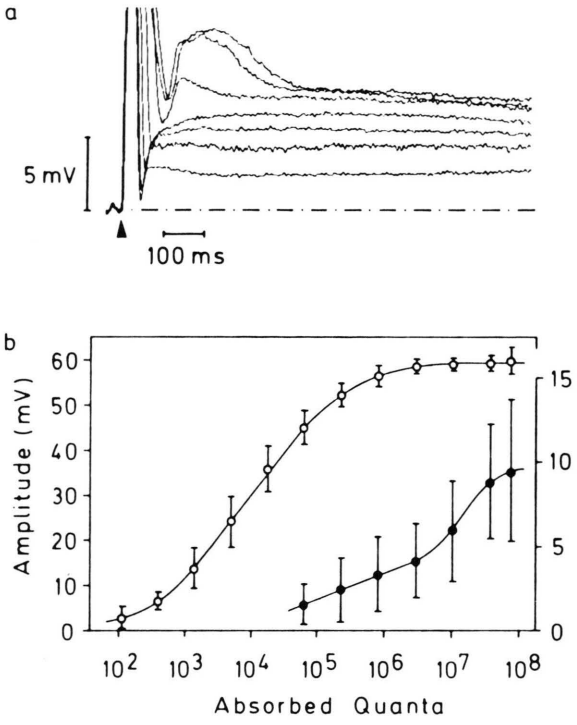

Fig. 2. a) Afterdepolarizations evoked by light flashes (arrowhead) of increasing intensity (interstimulus interval: 10 s). Resting potential: −52 mV; peak response truncated due to high gain. The composition of the superfusion medium was: 130 mM NaCl, 10 mM $CaCl_2$, 10 mM Hepes, pH = 7.0. The intensity of the flashes is shown in b. b) Amplitudes of the peak response (open circles, left ordinate) and of the afterdepolarization (closed circles, right ordinate) in relation to stimulus intensity. Data points are the mean of 5 experiments (bars: standard deviation).

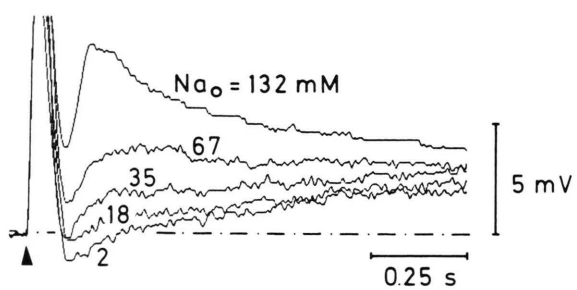

Fig. 3. Dependence of the afterdepolarization on the Na^+ concentration in the superfusion medium. Preparation alternately superfused with a Na^+ saline (Na_o = 132 mM) and with salines in which Na^+ was partially replaced by Li^+ to yield Na^+ concentrations of 67, 35, 18 and 2 mM. Ca^{2+} concentration (10 mM) was kept constant. The responses were recorded 40 s after the solution exchange. Data obtained from two preparations: trace at Na_o = 132 mM is the average of 10 responses; remaining traces are the average of 2 responses. Flash stimulus (arrowhead) led to 3×10^7 photon absorptions; peak response truncated due to high gain.

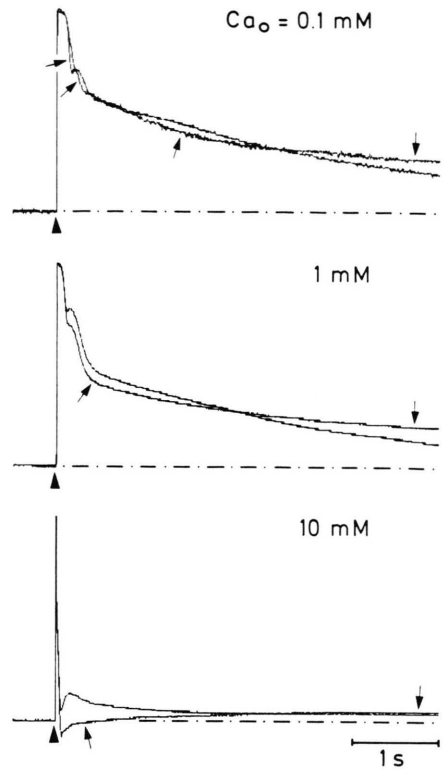

Fig. 4. Significance of Na^+ for the afterdepolarization depends on Ca_o. Na^+ (130 mM) was repetitively exchanged for Li^+ and *vice versa* at a constant Ca_o of 0.1 mM, 1 mM or 10 mM. Traces are the average of 3 (0.1 mM), 9 (1 mM) and 6 (10 mM) responses recorded 30 s after the exchange (3 different preparations). Before averaging the responses were normalized to peak; responses obtained in Li^+ marked by arrows. Arrowheads indicate light stimulus (3×10^7 photon absorptions). Mean amplitudes in Na^+ were: 43 mV (Ca_o = 0.1 mM), 47 mV (1 mM) and 60 mV (10 mM); corresponding values in Li^+: 40 mV, 43 mV and 51 mV.

3 mM (not shown), and 1 mM these afterdepolarizations also changed reversibly within less than 30 s: in the time range of up to 3 s after the stimulus the amplitude was reduced, whereas it increased at longer intervals. As seen in Fig. 4 the changes caused by the replacement of Na^+ decreased with Ca_o; at Ca_o = 0.1 mM a Na^+-dependent component of the afterdepolarization was not found. It is noted that at low Ca_o the amplitude of

the peak response was significantly smaller than at high Ca_o, whereby the resting potential was not affected [19]. This difference is due to the fact that at high Ca_o the peak response to saturating stimuli overshoots the extracellular potential (by about 10 mV at Ca_o = 10 mM), whereas the response just reached the extracellular potential at low Ca_o (compare [21]).

As seen in Fig. 1a the amplitude of the peak response was only slightly reduced shortly after replacing Na^+ by Li^+. When the superfusion with the Na^+-free medium was continued, the peak response remained stable if Ca_o was low (0.1 mM), whereas it continuously decreased at high Ca_o (10 mM; Fig. 5). In both cases the response rapidly recovered upon restoration of the initial conditions. These results fully agree with observations made in the ventral photoreceptor of *Limulus* [10]. It is emphasized that a reproducible suppression of the peak response was found only when the intensity of the flash stimulus was relatively low (Fig. 5: 10^4 photon absorptions). At high intensities (10^6 photon absorptions or more) a substantial suppression of the response (< 20% of the amplitude in the Na^+ medium) was observed only in 5 out of a total of 16 experiments. In 3 experiments the amplitude was only slightly reduced (> 80%) and remained stable for more than 20 min, and in the remaining 8 experiments the response amplitude approached an intermediate level. However, independent of the extent of the suppression, the peak response always recovered almost completely when Li^+ was replaced by Na^+ again (not shown).

In contrast, the peak response to stimuli of extreme intensity was always suppressed when the preparations were superfused with 100 mM $CaCl_2$ (Fig. 6). The suppression proceeded continuously if the preparations were repetitively stimulated (Fig. 6a). However, if the solution was exchanged in darkness, the suppression occurred in a different way. As is evident from Fig. 6b and c, the response elicited by the first flash in the Na^+-free medium was only slightly smaller than that recorded before the exchange. However, the response to the second flash 10 s later was strongly reduced (Fig. 6c) or even abolished (Fig. 6b). This effect is most probably due to the influx of Ca^{2+} during the response to the first flash. Surprisingly, the responses to the

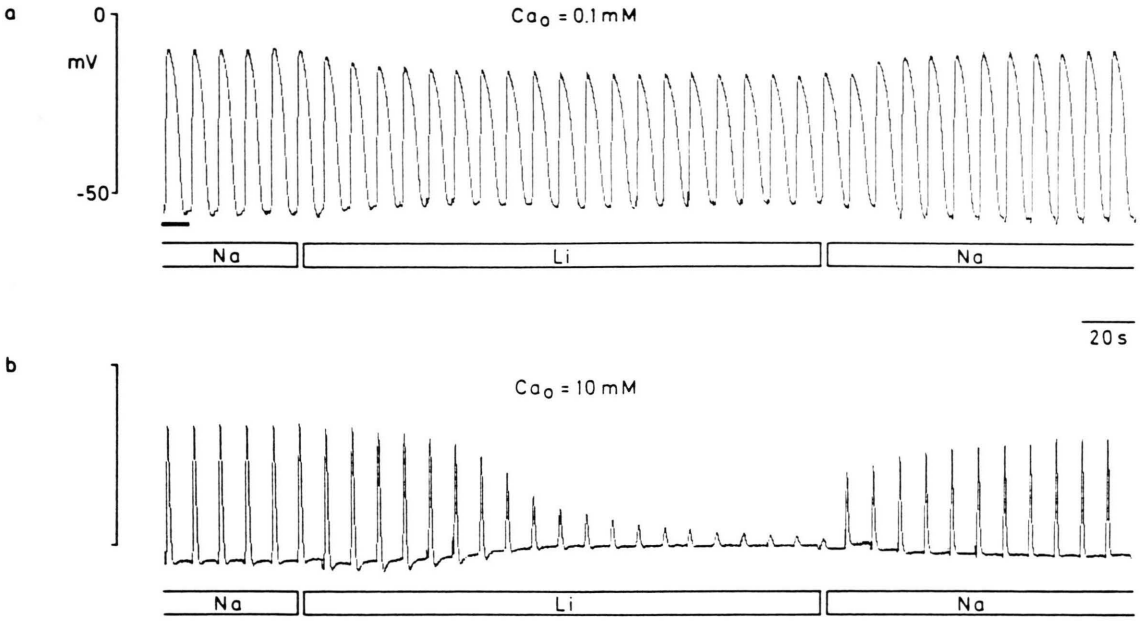

Fig. 5. Suppression of the peak response depends on Ca_o. a) Effect of replacing Na^+ by Li^+ on the peak response at Ca_o = 0.1 mM. Preparation repetitively stimulated by light flashes (10^4 photon absorptions; interstimulus interval 10 s). Responses recorded over a time range of 100 ms (bar beneath the 1. response) after the light flash, during the following 9.900 ms the registration was interrupted until to the next flash. b) Some experiment as in a, 3 min after increasing Ca_o to 10 mM.

following flashes increased progressively to a maximum, declined again and finally reached a constant amplitude that was about the same as in experiments in which the preparation was repetitively stimulated. It should be noted that in 3 out of a total of 7 experiments the amplitude of the first response after Na^+ removal was reduced by more than 50%, but also in 2 of these experiments the responses to the following stimuli were essentially suppressed and increased later again. In contrast to the suppression of the peak response its recovery appeared to be independent of light stimulation (Fig. 6).

Very similar effects were observed when Na^+ was replaced by Sr^{2+}, *i.e.*, a fast suppression of the afterdepolarization was followed by a delayed reduction of the peak response. Furthermore, both response components rapidly recovered upon restoration of the initial conditions (Fig. 7a). The regeneration of the response crucially depended on the presence of Na^+ (Fig. 7c) and was also observed when the superfusion medium contained Sr^{2+} instead of Ca^{2+} (Fig. 7b,c).

When Na^+ was replaced by Ba^{2+}, the peak response was also reversibly suppressed (Fig. 7d). However, both the suppression and, in particular, the regeneration of the response occurred clearly slower than in the presence of Ca^{2+} and Sr^{2+}. Furthermore, after restoration of the initial conditions the peak response was temporarily further suppressed or even abolished before the response began to recover (open arrow in Fig. 7d).

Completely different effects were found when Na^+ was replaced by Mg^{2+} (Fig. 7e): the response amplitude was only slightly reduced (by approx. 20%), and the duration of the response was con-

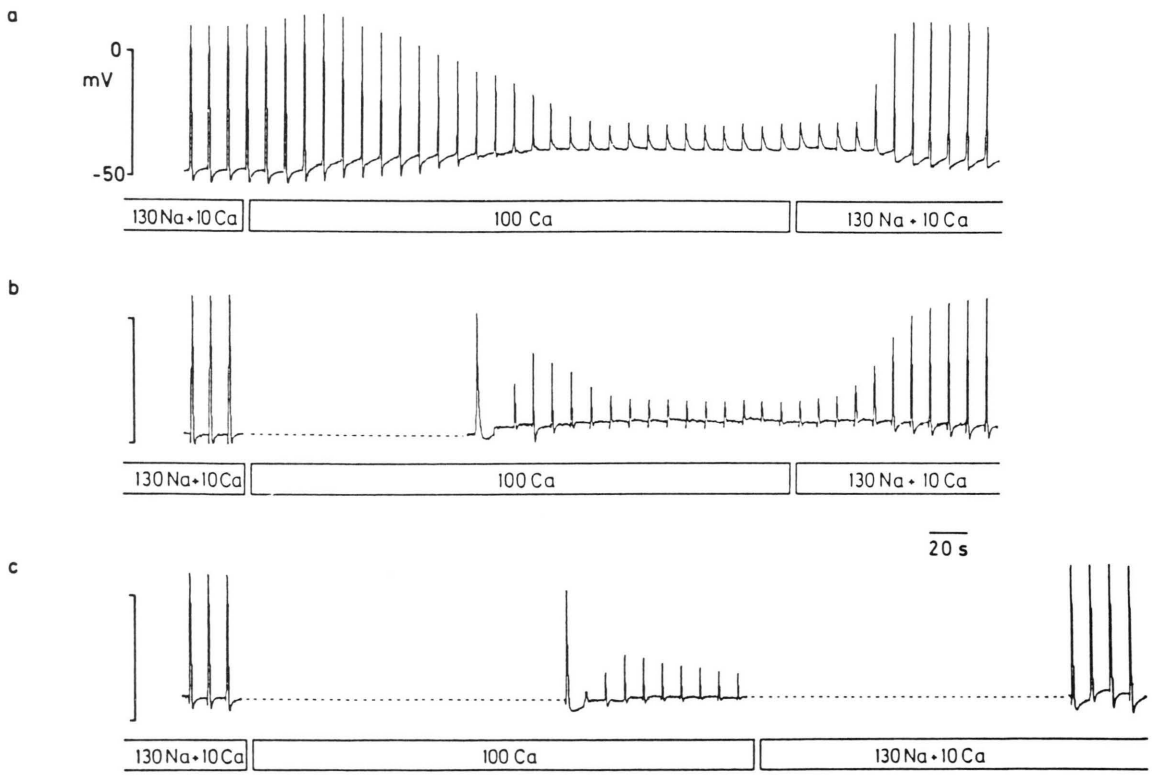

Fig. 6. Reversible suppression of the peak response upon transient replacement of Na^+ by Ca^{2+} and dependence of this effect on light stimulation. Ion concentrations are given in mM. Experimental protocol was the same as in Fig. 5, except that the flash stimulus led to 2×10^6 photon absorptions. Recordings in b and c were from the same cell. a) Preparation repetitively stimulated every 10 s. b) As a, except that Na^+ was replaced by Ca^{2+} in darkness. Repetitive stimulation was continued 2 min after exchanging the solution. c) As b, except that the first dark period was about 3 min and that the reverse exchange of Ca^{2+} for Na^+ was also performed in darkness.

siderably increased (half-width in 130 mM NaCl, 10 mM $CaCl_2$: 15 ms, after 8 min in 100 mM $MgCl_2$: 110 ms). The responses were constant for 10 to 15 min, but then the cells irreversibly depolarized within a few min. It is noted that even in the presence of 100 mM $MgCl_2$ effects of light and dark adaptation were observed. Thus after a dark interval of 3 min, the duration of the response was clearly longer than that of the responses recorded at repetitive stimulation before and after the dark interval (arrowhead in Fig. 7e).

Discussion

Several different processes may contribute to the afterdepolarization of invertebrate photoreceptors. Thus, in a variety of species it has been shown that the excess production of metarhodopsin by intense stimulation leads to a delayed activation of light-dependent channels which gives rise to a "prolonged depolarizing afterpotential" (PDA; for review see [22, 23]). Furthermore, changes in the transmembrane ion gradients may be involved, particularly a decrease in the electrochemical gradient of K^+. A light-induced increase in the extracellular concentration of K^+ (K_o) has been demonstrated in the retina of the honey-bee drone [13, 24, 25], and in flies an efflux of K^+ is suggested by the electric response of the pigment cells [26]. Finally, electrogenic ion transporting systems may participate in the afterdepolarization. Insect photoreceptors contain at least two electrogenic transport systems that are activated follow-

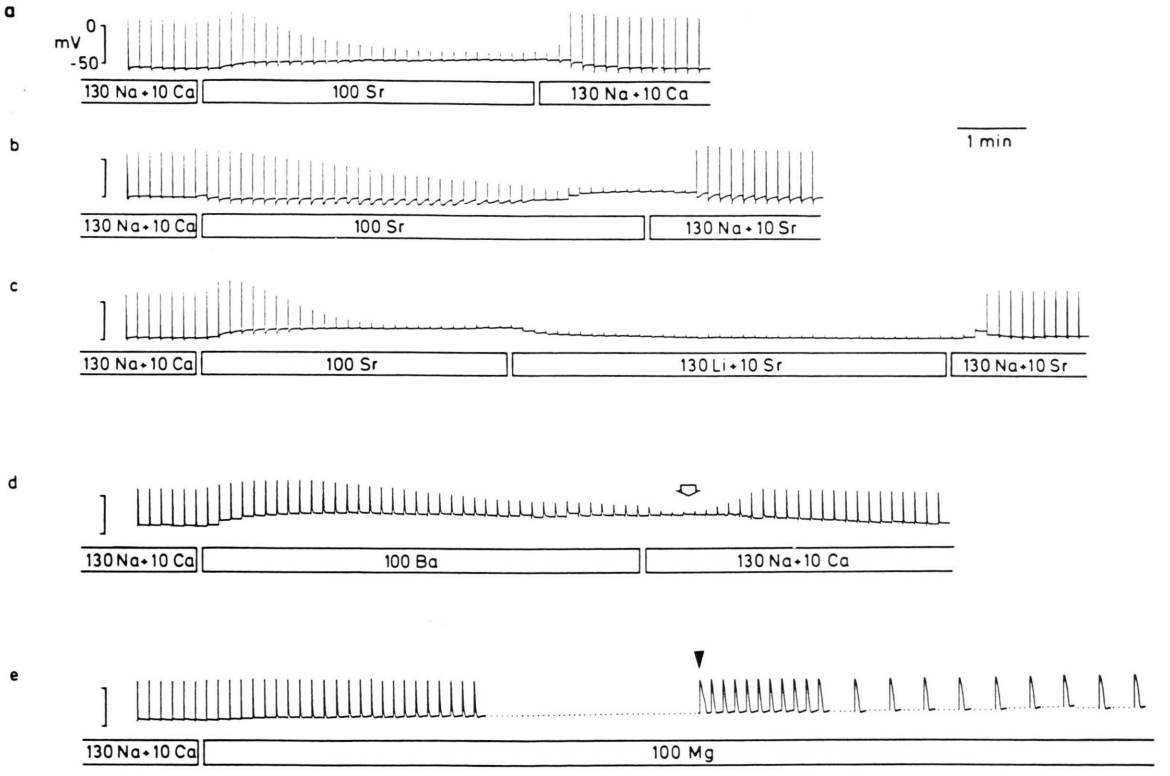

Fig. 7. Effect of replacing Na^+ by Sr^{2+} (a, b, c), Ba^{2+} (d) or Mg^{2+} (e) on the peak component of the photoreceptor response. Ion concentrations are given in mM. Experimental protocol was the same as in Fig. 5, except that the stimulus led to 2×10^6 photon absorptions and that the responses were recorded over 400 ms. Traces a, b and c were recorded from the same cell. In a to d, the preparations were stimulated every 10 s. In e, the stimulation was interrupted for 3 min, and after a sequence of 11 flashes, the interstimulus interval was increased to 30 s. Open arrow in d marks the further transient suppression of the response after replacing Ba^{2+} by Na^+. In e, arrowhead marks the first response after the dark interval which is clearly prolonged as compared to the subsequent responses or, respectively, to those recorded before the dark interval.

ing illumination: a Na^+ pump [27–29] and a Na^+-Ca^{2+} exchanger [13, 14, 17, 18].

In this report it is shown that the afterdepolarization of fly photoreceptors is reduced upon substitution of the extracellular Na^+. At Ca_o = 10 mM the afterdepolarization was completely abolished whereas the peak response was essentially maintained, which suggests that the two response components are due to different mechanisms. This interpretation is supported by the result that the occurrence of the afterdepolarization is strictly Na^+-dependent, since its suppression was independent of whether Li^+, choline or tris were used as substitute for Na^+ (Fig. 1b). In contrast, the light-dependent conductance of invertebrate photoreceptors is not Na^+-specific but is permeable for other monovalent cations as K^+ [10, 19, 30], Li^+ [10, 31] or even choline [10]. Therefore, the reduction of the afterdepolarization is unlikely to be the consequence of a reduced influx of positive charges through light-dependent ion channels which are activated during the afterdepolarization. This conclusion is in line with the result that at low Ca_o the afterpolarization was less or unsignificantly affected by the removal of Na^+ (Fig. 4).

The latter finding makes it also unlikely that the suppression of the afterdepolarization observed at high Ca_o is due to a reduced increase in K_o. Rather, the substantial maintenance of the peak response suggests that the light-induced efflux of K^+ is hardly affected by the removal of Na^+. Furthermore, after a transient replacement of Na^+, the afterdepolarization always reappeared, independent of whether the peak response was completely regenerated or not. On the other hand, an increase in K_o may well explain the large afterdepolarizations observed at low Ca_o (Fig. 4), since the duration of the peak response, *i.e.*, the time span over which an efflux of K^+ most probably occurs considerably increases when Ca_o is reduced (see [19]).

The reduction of the afterdepolarization might be due to the missing influx of Na^+ into the cell. In fly photoreceptors, the light-induced Na^+ influx probably activates the Na^+ pump [28]. Since the Na^+ pump transports 3 Na^+ out of the cell and 2 K^+ into it [32], the activation of the pump has a hyperpolarizing effect [29]. Consequently, if in the absence of Na^+ the pump is not activated, the amplitude of the afterdepolarization should increase. However, the afterdepolarization decreased upon Na^+ removal, and therefore a change in Na^+ pump activation cannot be the reason for the reduction of the afterdepolarization.

In contrast to the Na^+ pump, the activation of the Na^+-Ca^{2+} exchange system has a depolarizing effect, because in the forward mode this system transports 1 positive net charge per reaction cycle into the cell (3 Na^+: 1 Ca^{2+} or 4 Na^+: 1 Ca^{2+}/1 K^+; [1–5]). Furthermore, the result that the occurrence of the component strictly depends on the presence of extracellular Na^+ agrees well with the high Na^+ specificity of the Na^+-Ca^{2+} exchange system [1, 2]. Thus, both polarity and Na^+ specificity strongly suggest that the Na^+-dependent component of the afterdepolarization is due to electrogenic Na^+-Ca^{2+} exchange operating in the forward mode.

The activation of Na^+-Ca^{2+} exchange implies that the balance between the electrochemical gradients of Na^+ and Ca^{2+} is disturbed. Since illumination leads to the influx of Na^+, *i.e.* to a reduction in the driving force that fuels Ca^{2+} efflux, an outward transport of Ca^{2+} is only possible if the reduction in the Na^+ gradient is overcompensated by a reduction in the Ca^{2+} gradient, most probably by an increase in Ca_i. Such an increase may be caused by a Ca^{2+} influx from the extracellular space (see [11, 13, 14, 16]). In flies there is no evidence for Ca^{2+}-specific channels but Ca^{2+} may flow through the light-dependent channels, which are probably permeable for divalent cations. Thus, upon prolonged superfusion with 100 mM $MgCl_2$ the receptor responses were substantially preserved (Fig. 7e), which strongly suggests that these responses are due to the influx of Mg^{2+}. Furthermore, the marked responses recorded in 100 mM $CaCl_2$ (Fig. 6b, c) as well as the rather slow suppression of the responses in 100 mM $CaCl_2$ (Fig. 6a), $SrCl_2$ and $BaCl_2$ (Fig. 7) suggest that also Ca^{2+}, Sr^{2+} and Ba^{2+} can pass the light-dependent channels. In addition to a Ca^{2+} influx the release of Ca^{2+} from intracellular stores may contribute to an increase in Ca_i. In *Limulus* ventral photoreceptors light triggers the release of Ca^{2+} from intracellular stores [7, 33], and there is strong evidence that such a release also occurs in insect photoreceptors [13–15]. That no Na^+-dependent component was found at low Ca_o may suggest that an intracellular Ca^{2+} release is negligible, but this must not be the case at high Ca_o, since the Ca^{2+} content of the intracellular stores most probably depends

on Ca_o [15, 34]. At present it is not possible to estimate the relative contributions of Ca^{2+} influx and Ca^{2+} release to the increase in Ca_i which activates Na^+-Ca^{2+} exchange.

The depolarizing effect of the Na^+-Ca^{2+} exchange depends on a variety of parameters like intra- and extracellular concentrations of Na^+ and Ca^{2+} [1, 2, 35], membrane potential [36, 37] as well as the conductance of the plasma membrane for other ion species. In fly photoreceptors most of these parameters are not well defined and therefore, the relation between the afterdepolarization and the extracellular concentrations of Na^+ and Ca^{2+} cannot be interpreted unambiguously. Nevertheless, the results shown in Fig. 3 and 4 are consistent with the view that the Na^+-dependent component of the afterdepolarization is caused by electrogenic Na^+-Ca^{2+} exchange.

A contribution of electrogenic Na^+-Ca^{2+} exchange to the response of invertebrate photoreceptors has been demonstrated for the first time by showing that hypoxia or metabolic inhibitors induce a "transient post-illumination afterpotential" (TA) that is strongly dependent on the electrochemical gradients of Na^+ and Ca^{2+} [17, 18]. The results presented in this report extend these observations by showing that Na^+-Ca^{2+} exchange contributes to the receptor response also under normal conditions. As the afterdepolarization described here (Fig. 1, 3), the TA observed under metabolic inhibition was selectively suppressed by reducing the transmembrane gradient of Na^+. However, the afterdepolarization proceeded distinctly faster than the TA, and when Ca_o was reduced the TA increased [18], whereas the afterdepolarization decreased (Fig. 4). It is nearby to attribute these differences to changes in the Ca^{2+} release and uptake processes caused by the metabolic inhibition.

The reduction of Na_o decreases the driving force for the outward transport of Ca^{2+}. Consequently, the transport direction should reverse and in turn lead to an increase in Ca_i. This expectation has been verified in the ventral photoreceptor of *Limulus* [12]. Since a rise in Ca_i lowers the sensitivity of *Limulus* photoreceptors [6, 7] the replacement of extracellular Na^+ should cause a reduction in the receptor response, and indeed this has been shown in earlier studies [10, 38]. In agreement with the results in *Limulus*, the peak response of fly photoreceptors was suppressed when extracellular Na^+ was removed (Fig. 5, 6). Obviously, after reactivation of the exchange system by extracellular Na^+, the excess Ca^{2+} is rapidly pumped out of the cell, as evident from the fast recovery of the peak response. In particular, this latter result illustrates the significance of electrogenic Na^+-Ca^{2+} exchange for the functioning of fly photoreceptors.

Some observations, however, point to other mechanisms being involved in the control of Ca_i. Thus, the partial recovery of the peak response seen in Fig. 6 b, c suggests that a decrease in Ca_i also occurs in the absence of extracellular Na^+. Since in 100 mM $CaCl_2$ an outward transport of Ca^{2+} by Na^+-Ca^{2+} exchange can be excluded, the intracellular Ca^{2+} must either be sequestered by intracellular organelles [39] or pumped out of the cell by a Na^+-independent transport system, *e.g.* an ATP-driven Ca^{2+} pump [40]. Ca^{2+} pumping and/or sequestration may also explain why the replacement of Na^+ by Li^+ was less effective in suppressing the peak response to intense stimuli than the replacement by 100 mM $CaCl_2$, as well as the finding that the suppression of the response proceeded slower than its recovery (Fig. 5, 6).

A continuous suppression of the peak response was also observed when Na^+ was replaced by Sr^{2+} or Ba^{2+}, whereas replacement by Mg^{2+} left the response essentially unchanged for a longer period (Fig. 7). These findings are in accord with experiments in the ventral photoreceptor of *Limulus*, which showed that the injection of Ca^{2+}, Sr^{2+} or Ba^{2+}, but not that of Mg^{2+}, had a desensitizing effect [31]. Since the transient replacement of Na^+ by Sr^{2+} induced very similar effects as the replacement by Ca^{2+}, the Na^+-Ca^{2+} exchange system of fly photoreceptors is obviously not specific for Ca^{2+}, but also transports Sr^{2+}. This conclusion agrees well with results obtained in cardiac muscle cells [41–44]. Concerning the transport of Ba^{2+} by the Na^+-Ca^{2+} exchange mechanism the literature is contradictory [42, 43]. In fly photoreceptors, the effects that were observed when Na^+ was replaced by Ba^{2+} give no clear evidence for Ba^{2+} being transported by the Na^+-Ca^{2+} exchange system. Particularly, the further transient suppression of the receptor response after restoration of the initial conditions (Fig. 7 d) shows that some aspects of the action of Ba^{2+} are qualitatively different from those of Ca^{2+} or Sr^{2+}.

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