The Nature of Rhodopsin-Triggered Photocurrents in *Chlamydomonas*. II. Influence of Monovalent Ions

Christina Nonnengässer,* Eva-Maria Holland,‡ Hartmann Harz,* and Peter Hegemann*‡
*Max-Planck-Institut für Biochemie, 82152 Martinsried, and ‡Institut für Biochemie I, 93040 Regensburg, Germany

ABSTRACT *Chlamydomonas* exhibits a sequence of a photoreceptor current and two flagellar currents upon stimulation with bright green flashes. The currents are thought to be a prerequisite for the well-known photophobic responses. In the preceding paper, we analyzed the kinetics of these currents and their dependence on extracellular divalent ions. Here, we show that the photoreceptor current can be carried by monovalent ions ($K^+ > NH_4^+ > Na^+$), provided that the driving force is high enough. The small residual photoreceptor current observed in the absence of Ca^{2+} is able to evoke flagellar currents at low extracellular pH. This demonstrates that signal transduction from the rhodopsin to the flagella is not inevitably dependent on extracellular Ca^{2+}. Double-flash experiments exclude a contribution of intra-rhodopsin charge movements to the photoreceptor current signal. Evidence will be provided for the existence of nonlocalized K^+ outward currents, which counterbalance the localized Ca^{2+} influx and repolarize the cell after a light flash. A model is presented that explains the different pathways for direction changes and phobic responses.

INTRODUCTION

Rhodopsin-mediated photoreceptor (P) currents and flagellar (F_F and F_S) currents in *Chlamydomonas* can be measured with a suction pipette technique under different configurations, i.e., with eyespot or flagella inside or outside the pipette (Harz and Hegemann, 1991). In the preceding paper (Holland et al., 1996) we presented a further characterization of these currents. The full-sized P current was recorded with a small patch pipette placed directly over the eyespot. It became obvious that the P current appears with a delay of less than 50 μs. This extremely fast channel opening represents a close coupling between rhodopsin and the channel. Experiments carried out in the suction mode demonstrated the complex Ca^{2+} dependence of the P current, but they also revealed that small P currents are still detectable in the absence of any Ca^{2+}. To explain the residual P current in *Haematococcus*, Sineshchekov (1991) argued that a charge movement within the rhodopsin molecules is similar to that which appears during the early receptor potential found in animal visual processes. Harz et al. (1992) explained the residual current in *Chlamydomonas* by a Ca^{2+}-independent current carried by monovalent ions. Full-sized P currents appear by the replacement of Ca^{2+} by Sr^{2+} and Ba^{2+}, whereas Mg^{2+} appeared to be inhibitory. In Ba^{2+} the F_F currents and especially F_S currents were enlarged, which leads to extended spiraling of free-swimming cells. Although these suction experiments enabled physiologically relevant photocurrents to be detected, the determination of the membrane potential in these algae is still impossible. Conclusions on the membrane potential or relative membrane potential changes can only be drawn indirectly from the observed photocurrents or from experiments with membrane-permeable dyes (Malhotra and Glass, 1995). So far, all identified currents are depolarizing currents and nothing is known about repolarization after a flash or in continuous light.

Besides Ca^{2+}, K^+ is also essential for a *Chlamydomonas* cell to perform behavioral light responses. A K^+ contribution to photocurrents is expected but has not yet been demonstrated. Here the characterization of the photocurrents is extended by analyzing the influence of monovalent ions, with a special focus on K^+ and low pH.

Received for publication 21 September 1994 and in final form 12 November 1995.

Address reprint requests to Dr. Peter Hegemann, Institut für Biochemie I, Universität Regensburg, Universitätsstraße 31, Postfach 397, 93040 Regensburg, Germany. Tel.: 0049-941-943-2814; Fax: 0049-941-943-2936; E-mail: peter.hegemann@biologie.uni-regensburg.de.

The present address of Dr. Harz is Botanisches Institut der Ludwig-Maximilians-Universität München, Menzinger Straße 67, 80638 München, Germany.

0006-3495/96/02/932/07 $2.00

MATERIALS AND METHODS

All experiments were carried out with the cell-wall-deficient mutant CW2.

Cells were grown and differentiated into gametes as described (Holland et al., 1996).

Photocurrents were measured as described by Harz et al. (1992) with the modifications described by Holland et al. (1996).

In most experiments NMG^+/K^+ buffer was used (5 mM HEPES, 9 mM HCl, 1 mM KCl, 200 μM BAPTA (1,2-bis(2-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid, tetrapotassium salt) adjusted with *N*-methyl-D-glucamine (NMG) to pH 6.8). Because the NMG^+/K^+ buffer contained Ca^{2+} in the micromolar range due to impurities of the reagents, measurements at low Ca^{2+} concentrations were carried out in KP_i buffer (3 mM K_2HPO_4, 200 μM BAPTA, adjusted with HCl to pH 6.8; final Cl^- concentration was near 2 mM), which was prepared from "suprapur" reagents (Merck, Darmstadt). Ca^{2+} or Ba^{2+} were added as $CaCl_2$ or $BaCl_2$. The total Ca^{2+} concentration required for a defined concentration of free Ca^{2+} was calculated as described by Holland et al. in the accompanying paper (1996).

Model for recording localized and nonlocalized currents

Before we present experimental details, we would like to remind the reader how localized and nonlocalized currents or combinations of both are detected

in this unicellular alga with suction electrodes (Fig. 1). Localized inward currents always depolarize the cell. The depolarization is accompanied by a compensatory charge movement at the outer surface of the plasmalemma due to the capacitor properties of the membrane. When the ion-conducting region is in the pipette (*a*, flagella inside), the inward current itself is recorded. When the regions conducting the inward currents are outside the pipette (*b*), only displacement currents can be detected. The direction of these displacement currents is inverted and thus they are shown as positive current signals. Displacement currents are composed of negatively and positively charged ions. Their relative contribution depends on their concentration and mobility. The detectable current signal is proportional to the part of the cell surface in the pipette and thus in configuration (*b*) is always smaller than that under configuration of (*a*). The maximum inward current is observed when the source region is in the pipette with only a small portion of the plasmalemma. Otherwise, an increasing capacitive current diminishes the inward current (calculated in Harz et al., 1992). When a localized cation influx is coupled to a nonlocalized cation efflux (*c*), only that part of the outward current is detected which flows into the pipette. The current passing over the rest of the plasmalemma back into the bath remains undetected. No capacitive currents are involved if the inward current equals the outward current. Because capacitive currents (*a*) and (*b*) and the nonlocalized cation efflux (*c*) have the same direction, their relative contribution cannot be determined unless asymmetrical ionic conditions are realized, i.e., different ion concentration in bath and pipette. Only this would enable a distinction between (*b*) and (*c*) to be made. In any case, the detected currents are defined fractions of the localized inward currents. Nonlocalized outward currents (*d*) are not detectable under symmetrical conditions because they are completely counterbalanced by a capacitive ion movement at the membrane. Evenly distributed channel activities can be analyzed, only when the ionic conditions are asymmetrical.

RESULTS

Influence of monovalent cations on the transient P and F_F currents

The question of how monovalent ions contribute to the transient photocurrents and to signaling in *Chlamydomonas* was tested by replacing K^+ by other monovalent ions. When at 100 μM Ca^{2+}, K^+ is replaced by Na^+ or NH_4^+, the P current and F_F currents are kept unchanged. When K^+ was simply omitted from the medium, again no immediate changes of the photocurrents were observed. Both results stand against a contribution of K^+ to any of the photocurrents under physiological conditions

Because the membrane potential of the alga must be close to the K^+ equilibrium potential (E_K), both channels might conduct K^+ when the driving force for K^+ is increased. The increase of extracellular K^+ to 20 or 40 mM enlarged the P and F_F peak current amplitudes and accelerated the decay kinetics. This indicates that K^+ is indeed conducted by both light-activated channels when the driving force is high (Fig. 2 *a*). The relative conductance of the P current for other monovalent ions is smaller, with a relative preference of $K^+ > NH_4^+ > Na^+$. The broader F_F signal at high NMG^+ (Fig. 2 *a*) is not due to fluctuation of its time of appearence in individual traces, but rather resembles an altered on-and-off kinetic that is similar to that which was observed in the presence of Mg^{2+} (Holland et al., 1996).

If K^+ is conducted by the same P channel as Ca^{2+}, it must compete with Ca^{2+}. Consequently, extracellular high K^+ should have a more dramatic effect in the absence of Ca^{2+}. Unfortunately, at low Ca^{2+} and high K^+, spontaneous electrical events are observed that reduce the size of the P current if it precedes the flash by less than 200 ms.

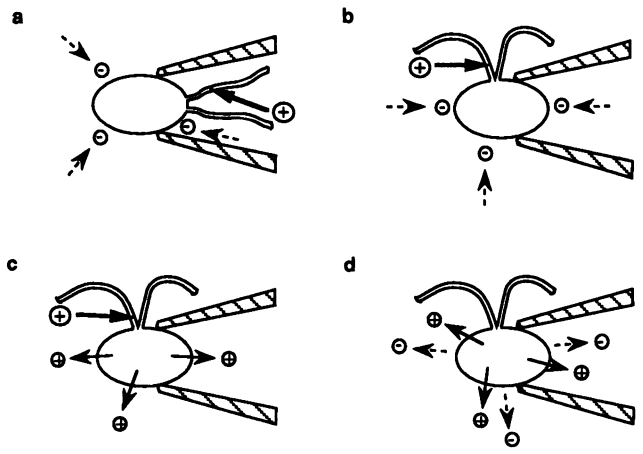

FIGURE 1 Four principal ionic events that are relevant for photoresponses in *Chlamydomonas*. A pure localized inward current exemplified by the fast flagellar current (F_F) at two configurations with flagella inside (*a*) and outside (*b*) the pipette. The current depolarizes the cell and thus it is accompanied by a displacement current onto the cell. (*c*) Combination of a localized inward current and a nonlocalized outward current. Only the part of the outward current that is directed into the pipette is detectable. (*d*) A nonlocalized cation outward current is completely cancelled by the displacement current and thereby is not detectable. Localized inward currents are symbolized by bold arrows, nonlocalized outward currents by faint arrows, and capacitive displacement currents by dashed arrows.

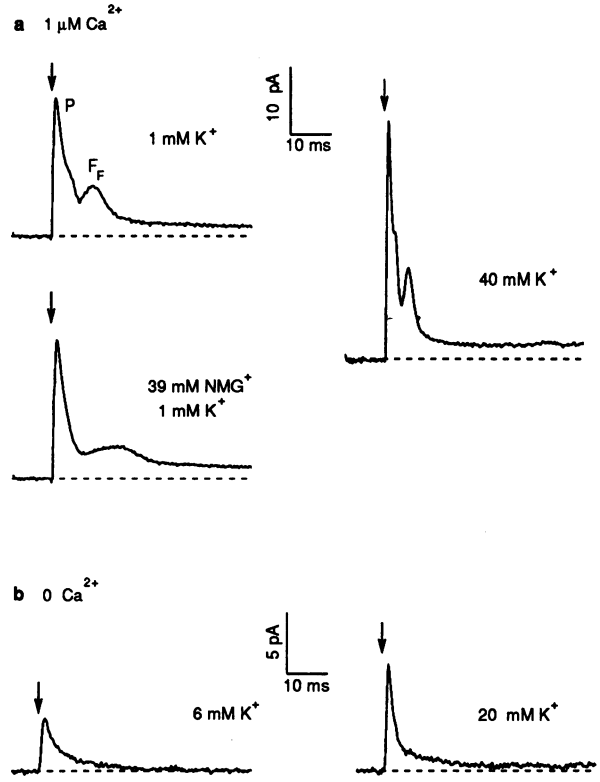

FIGURE 2 Influence of high extracellular K^+ and NMG^+ on the P and F_F current at 1 μM Ca^{2+} (*a*) and the influence of K^+ on the P current in absence of Ca^{2+} (*b*).

However, several experiments demonstrated that K^+ increases the residual Ca^{2+}-independent current (Fig. 2 *b*). However, the K^+-induced increase still has the same magnitude as that found in presence of Ca^{2+}. In *Chlamydomonas*, Ca^{2+} apparently does not greatly block the K^+ conductance as it does in most mammalian Ca^{2+} channels. Setting extracellular K^+ to zero in the absence of Ca^{2+} does not completely suppress the current or switch the current negatively. This finding yields two conclusions. First, the K^+ conductance of the P channel must be inwardly rectifying. Second, ions other than K^+ must carry the residual P current in the absence of Ca^{2+} and K^+. A proton influx or an anion efflux must be considered, because NMG^+ was the only cation in the medium in these experiments.

Double-flash experiments

In addition to H^+ or anions, charge movements caused by conformational changes within the rhodopsin may also be responsible for a Ca^{2+}-independent residual P current (Sineshchekov et al., 1990). This hypothesis has been tested by applying two consecutive flashes with a short time delay of 100 ms. Under all conditions in which flagellar currents appeared in response to the first flash, the second flash showed no response at all. In the experiment presented in Fig. 3, flashes with a photon exposure of 3.5×10^{18} (*a*) and 1.5×10^{19} photons m^{-2} (*b*) produced photoreceptor peak currents of 10.7 and 21 pA. To draw conclusions from these double-flash experiments as to whether or not currents originate from light-induced charge movements within the rhodopsin, the number of rhodopsins bleached by both flashes had to be calculated first. Roughly 3.5% of the rhodopsin is bleached by 23% flash intensity (*a*) and 15% by a 100% flash (*b*), assuming an absorption cross section of 1.9×10^{-20} m^{-2} and a quantum efficiency of 0.67, which is typical for rhodopsin. In the absence of Ca^{2+}, the same flashes on the same cell produce residual currents of below 30%, i.e., ~2.6 and 5.2 pA, respectively. If these Ca^{2+}-independent currents reflect a charge movement within the rhodopsin, then a second flash with the same energy as the first should cause residual currents of at least 2.5 pA (*a*) and 4.4 pA (*b*). The complete disappearence of the P current by no means supports the claim that the residual current is caused by a charge movement within the rhodopsin. If this were the case, we would have to expect intracellular Ca^{2+} or the altered membrane potential to inactivate all the rhodopsin in the cell. This has not been observed in any rhodopsin system so far. Yet it also can be said that the disappearence is fully compatible with an inactivation of the P channel caused by the first flash. The interpretation of the residual Ca^{2+}-independent P current as an ionic current through the photoreceptor channel is further corroborated by the observation that all three photocurrents, including the P current, vanished in 1 mM La^{3+} (Fig. 4). We could not find any concentration at which F currents were reduced with a constant P current. Other than Cd^{2+} (Holland et al., 1996), La^{3+} inhibits the P current better than the F currents but, because F currents depend on the P current integral, all three photocurrent components disappear.

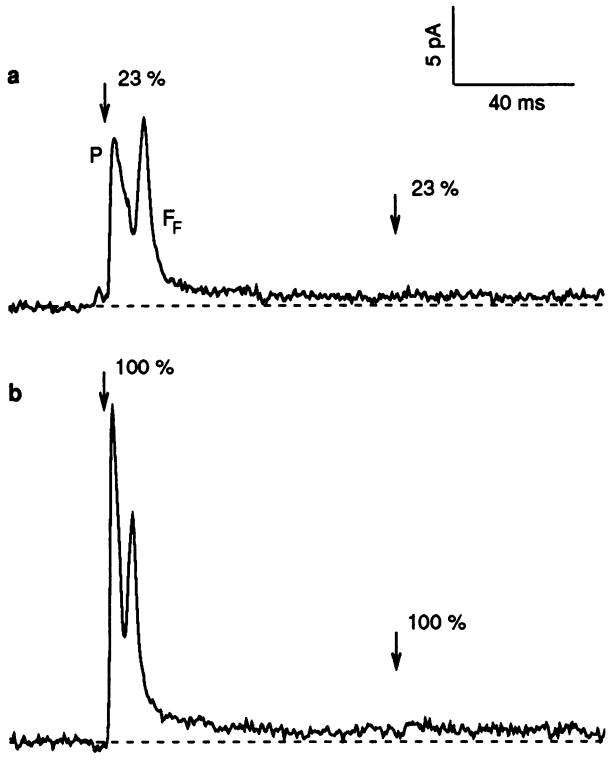

FIGURE 3 Photocurrents in response to double flashes of the same energy. The times of flash application are indicated by arrows. 23% and 100% indicate the flash energy. 100% flash energy corresponds to 1.5×10^{19} photons m^{-2}.

Flagellar currents in the absence of Ca^{2+}

As shown in Fig. 2 *b*, the residual P current cannot trigger any flagellar currents in a Ca^{2+}-free medium. The current interpretation, mainly drawn from earlier light titration experiments, is that small P currents cannot depolarize the cell far enough to reach the critical level where flagellar channels are activated. As shown by using membrane-permeable dyes, acidification of the bath medium depolarizes a *Chlamydomonas* cell (Malhotra and Glass, 1995). At low pH the cell should be sensitized for flagellar responses. Because the P current itself is sensitive to pH changes, the eyespot was kept in the pipette and the pH was lowered in the bath medium (Fig. 5). Ca^{2+}-independent F_F currents were detected at pH 5.8 or below. Their amplitudes were only one-third of those observed under standard conditions. Three conclusions can be drawn from these experiments. First, the experiment confirms that not only photoreceptor channels but also flagellar channels conduct ions other than Ca^{2+}. Second, activation of the F_F channel does not strictly depend on extracellular Ca^{2+}. Third, the data confirm that F_F channels are voltage activated.

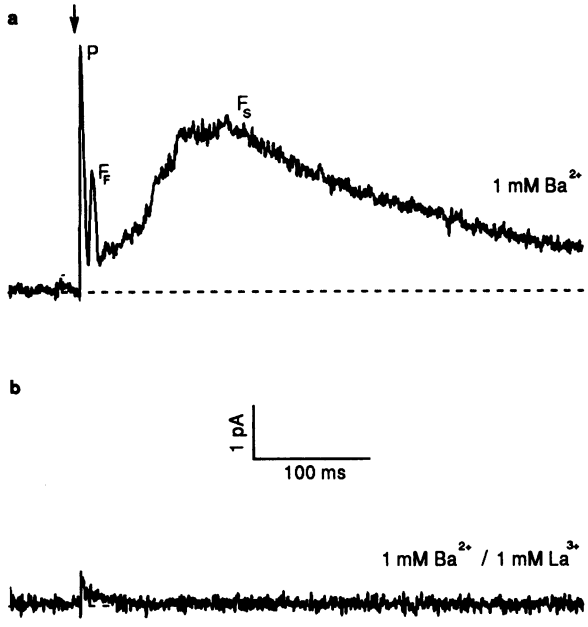

FIGURE 4 Photocurrents at 1 mM Ba^{2+} in the absence (*a*) and presence (*b*) of 1 mM La^{3+}.

The slow flagellar inward current F_S is accompanied by a K^+ outward current

As mentioned by Holland et al. (1996), F_S has a small amplitude of only 1 pA or less under standard conditions. However, it drastically increases when Ca^{2+} is substituted by Ba^{2+}. Under these conditions, the F_S current exhibits a current integral that is 20 times larger than those of the P and F_F current together. Assuming a specific membrane capacitance of 1 μF cm^{-2} and a cell surface of 200 μm^{-2}, the cell already depolarizes by about 100 to 200 mV during the P and F_F current (Harz et al., 1992). Thus, the large F_S current cannot reflect a pure Ba^{2+} influx. This would polarize the cell to completely unrealistic positive values. Therefore, F_S can only be understood as a localized Ba^{2+} influx that is accompanied by a simultaneous outward current. Because K^+ is another major cation in the standard medium in addition to Ca^{2+}, and the system is sensitive to high K^+ (Fig. 2), we assumed that the membrane potential in its resting state is close to E_K and that K^+ is the ion that is most likely to carry the ion efflux.

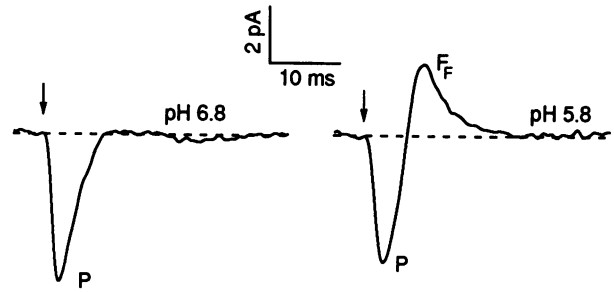

FIGURE 5 Flagellar currents in the absence of Ca^{2+}. Photocurrents, measured under a configuration with eyespot in the pipette, in the absence of Ca^{2+} at pH 6.8 or 5.8. The pH in the pipette was maintained at 6.8.

As previously discussed (Fig. 1), a coupling of ion influxes and effluxes can only be distinguished from a pure influx under asymmetrical ion conditions. To experimentally demonstrate a nonlocalized K^+ efflux, photocurrents were measured at 100 μM Ba^{2+} with varying K^+ inside and outside the pipette. Currents recorded under four basic ionic conditions are shown in Fig. 6. Under symmetrically low K^+ conditions (Fig. 6 *a*) with 1 mM K^+ inside and outside the pipette, a F_S is observed that decays within some hundred milliseconds. As we predicted, this long-lasting flagel-

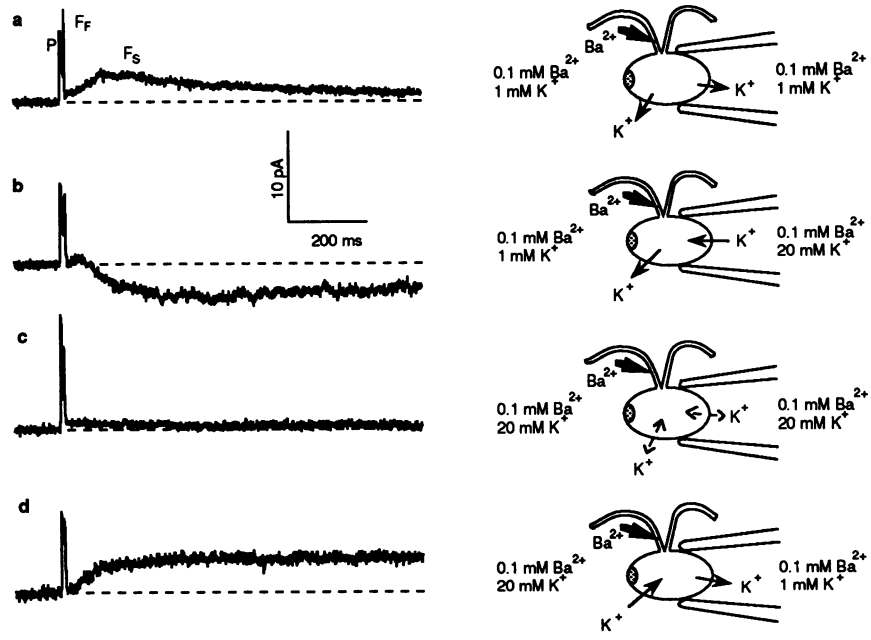

FIGURE 6 Photocurrents at 100 μM Ba^{2+} under four different K^+ conditions. The interpretation of ionic processes during the slow currents are schematized on the right.

lar current is made possible by a nonlocalized K^+ outward current that accompanies the flagellar inward current. Part of this coupled influx and efflux is measured as an outward current from the cell into the pipette. The major part of the K^+ current, however, returns to the bath solution and is electrically silent. It should be emphasized that the measured K^+ current is directly proportional to the Ba^{2+} inward current. Therefore, the recorded current was named F_S. The K^+ efflux enables the cell to increase the intraflagellar Ca^{2+} or Ba^{2+} concentration without any further depolarization. Furthermore, such a K^+ efflux can be used for repolarization of the cell toward the resting state either during or after the Ba^{2+} influx. As seen from individual recordings, F_S shows a significant fluctuation and a quite variable delay (Fig. 7), suggesting that only a few individual channels are involved.

When K^+ is raised in the pipette to 20 mM, a large negative current signal is recorded (Fig. 6 *b*). It is thought that there is a K^+ flux along the gradient from the pipette through the cell into the bath solution (transcellular K^+ current). Under these experimental conditions, the detected K^+ current is extended and does not reflect the Ba^{2+} inward current alone. So it should not be termed F_S. With high K^+ on both sides of the cell (symmetrical conditions), K^+ outward currents were totally suppressed (Fig. 6 *c*). At high K^+, the transient P and F_F currents are increased (Fig. 2), and the slow Ba^{2+} inward current is reduced almost to zero. This is the major piece of experimental evidence which supports the claim that a large Ba^{2+} inward current is only possible if it is coupled to a K^+ efflux.

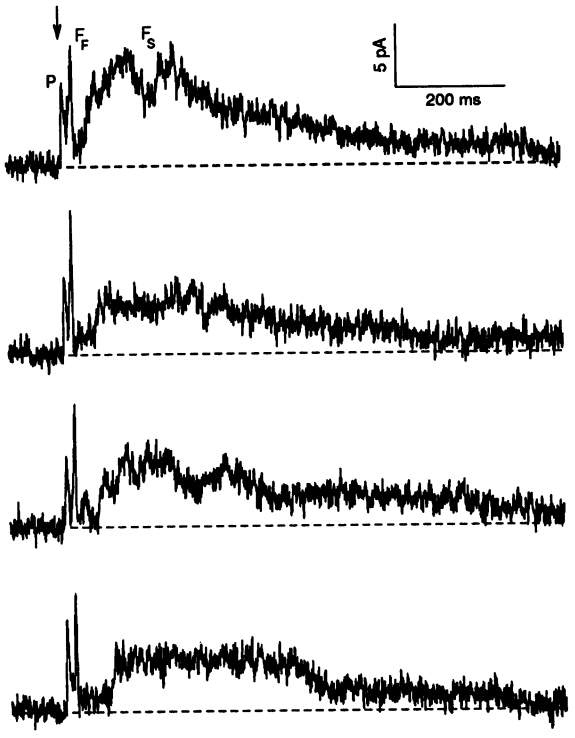

FIGURE 7 Individual traces of photocurrents recorded at 1 mM K^+ and 1 mM Ba^{2+}. Flash energy was 50%.

The fourth condition with high K^+ outside and low K^+ inside the pipette (*d*), i.e., the inverse gradient as in (*b*), shows another interesting result. Here, the transcellular K^+ current is drastically extended in its duration, whereas its amplitude is not significantly increased (Fig. 6 *d*). At 20 mM K^+ in the bath solution, the transcellular K^+ current sometimes persists for more than 8 s. This long-lasting current must again be interpreted as a transcellular K^+ flux. When the major part of the cell is exposed to high K^+, the cell cannot repolarize to normal values.

To further support the appearance of a transcellular K^+ current, we tried to suppress the K^+ current by extracellular application of Cs^+. Surprisingly, Cs^+ was inefficient up to 20 mM. Nonetheless, the existence of transcellular K^+ currents has been corroborated by the finding that large negative currents are also observed with asymmetrical K^+ in the presence of Ca^{2+}, i.e., under conditions in which the F_S inward current is small.

In summary, at low symmetrical extracellular K^+, the flagellar Ca^{2+} or Ba^{2+} influx triggers a nonlocalized K^+ efflux that accompanies the influx. At asymmetrical K^+, activation of the same K^+ channels leads to an unphysiological transcellular K^+ current that is fairly independent of the amount of divalant cations entering the flagella.

Ca^{2+} inactivates the slow flagellar current

The question of why F_S is so much larger in Ba^{2+} than in Ca^{2+} still remains. Because Ba^{2+} inhibits K^+ channels in other systems, it was conceivable that Ba^{2+} also blocks K^+ channels in *Chlamydomonas* that are exposed to the bath solution, whereas channels in the pipette are not blocked, so that the total K^+ efflux is directed into the pipette. However, experiments with Ba^{2+} or Ca^{2+} in the pipette gave the same results. This demonstrates that at least K^+ channels of the plasmalemma are not blocked. Perhaps blocking of flagellar K^+ channels could explain the enlarged Ba^{2+} current. A concomitant K^+ efflux together with a Ca^{2+} or Ba^{2+} influx would be electrically silent, i.e., it would diminish the F_S signal (short circuit). A blockage of such a flagellar K^+ channel would then favor K^+ efflux via the cell body and thereby increase the detected F_S amplitude. This question was answered by replacing Ca^{2+} by a 1:1 Ca^{2+}/Ba^{2+} mixture. F_S was small, as in pure Ca^{2+} (Fig. 8), excluding the possibility that the large Ba^{2+} current is caused by a Ba^{2+}-induced blockage of flagellar K^+ channels. The result strongly suggests that the low F_S amplitude in Ca^{2+} is caused by a Ca^{2+} induced down-regulation of the flagellar Ca^{2+} channels. This Ca^{2+} regulation is further confirmed by the finding that F_S saturates in Ba^{2+} at much higher concentrations of more than 1 mM, as it does in Ca^{2+}, where the largest current has already been recorded at 1 μM.

DISCUSSION

Although the photoreceptor current in *Chlamydomonas* seems to be mainly carried by Ca^{2+} under physiological conditions,

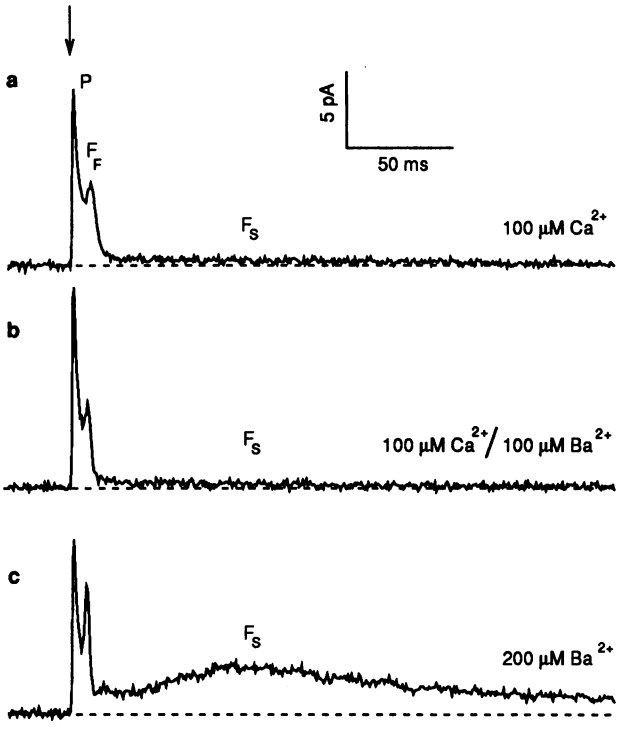

FIGURE 8 Photocurrents at 100 μM Ca^{2+} (*a*), 100 μM Ca^{2+}/100 μM Ba^{2+} (*b*), and 200 μM Ba^{2+} (*c*) in NMG^+/K^+ buffer.

the P channel is nevertheless unspecific. It permeates K^+, NH_4^+, and Na^+, and, in contrast to typical animal Ca^{2+} channels, Ca^{2+} does not suppress this K^+ conductance.

The larger peak amplitude and faster decay of the P currents at high K^+ compared to low K^+ provides an additional argument for a voltage-dependent inactivation. However, empirical evidence suggests that parameters other than voltage may influence P channel inactivation. For example, the P current inactivation is unexpectedly fast for a given peak amplitude in the absence of divalent ions. This results in smaller current integrals. One plausible explanation for this is the surface potential, which increases in the absence of divalent ions. This leads to a reduced transmembrane potential, which is sensed by the channels. So the P channel would inactivate at smaller P current integrals.

To explain the residual P current in the absence of Ca^{2+} and K^+, protons and anions have to be taken in account. Under physiological conditions, however, the contribution of protons or anions is probably very small. Sineshchekov's hypothesis that the residual current is caused by a charge movement within the rhodopsin molecules and thereby is similar to the early receptor potential of vertebrate eyes appears less likely to be true because of the presented double-flash experiments. A total charge movement on the order of 80 fQ during a residual P current (5×10^5 charges) (see Harz and Hegemann, 1992, for further details) clearly exceeds the number of the rhodopsin molecules in the range of only 10,000 molecules per CW2 cell (Smyth et al., 1988; Harz et al., 1992). If one considers the 36 lysines of the opsin sequence, Sineshchekov's argument cannot be easily dismissed because large charge movements could be realized. However, kinetic variation of the residual current with extracellular K^+ and current inhibition through La^{3+} is inconsistent with the interpretation that the residual P current represents a charge movement within the rhodopsin.

Because the photoreceptor inward current depolarizes the cell, the F_F channel must be activated at higher voltages (less negative than the resting potential). The constant P current integral between the flash and the beginning of the F_F current observerd at different K^+ concentrations and the activation of F_F channels at low pH in the absence of Ca^{2+} by smaller P currents support the conclusion that the membrane voltage activates the F_F channels. Under physiological conditions the F_F current seems to be a pure Ca^{2+} current. No evidence has been presented so far for a counterbalance of the Ca^{2+} influx by a K^+ efflux as it is manifested in case of the Ca^{2+}/K^+ ciliary action potentials in *Paramecium* (Eckert and Brehm, 1979) or in the case of the Na^+/K^+ action potential in metazoan neurons. In contrast, the Ca^{2+} or Ba^{2+} influx into the flagella during F_S is accompanied by a concomitant K^+ efflux distributed all over the plasmalemma. Although we argued that under resting conditions the membrane potential is near E_K, a K^+ efflux is not a contradiction, because the membrane is significantly polarized by the P and F_F currents toward positive values before this third current signal begins to appear. Opening of the slow flagellar channels together with K^+ channels allows the cell to reach and maintain a precise intracellular Ca^{2+} concentration without depolarizing to extreme positive values. For this precision a Ca^{2+}-mediated inactivation of the F_S current helps to account for large variations of extracellular Ca^{2+} concentrations with which the cells have to cope in their natural environment. This regulation is not possible in the presence of Ba^{2+} without Ca^{2+}. The K^+ current keeps the driving force for Ca^{2+} constant at a low level over a long time period. In Ba^{2+}, the K^+ conductance apparently begins at a low level after the two transient P and F_F currents have ceased, i.e., it is only slowly activated. Its rise nicely correlates with the increase of the slow current under symmetrical and asymmetrical conditions (Fig. 6). Beyond its importance for the Ca^{2+} influx, the K^+ outward current under physiological conditions can act for cell repolarizsation beginning either during or after the Ca^{2+} influx.

The current view of the rhodopsin-mediated ionic processes in *Chlamydomonas* is summarized in Fig. 9. The light receptor (chlamyrhodopsin) is closely coupled to a Ca^{2+} channel (P channel) that it activates upon light absorption within 50 μs. The presented experiments are consistent with the prevailing model that the P current depolarizes the cell within a few milliseconds in a graded manner with increasing light stimulus. In response to small flashes the P current may initiate some intracellular amplification of the Ca^{2+} signal by activating intracellular Ca^{2+} stores, enabling the released Ca^{2+} to induce brief direction changes. At the moment, however, there is no experimental method available for *Chlamydomonas* to address this problem. At high photon exposure, the P current serves as a

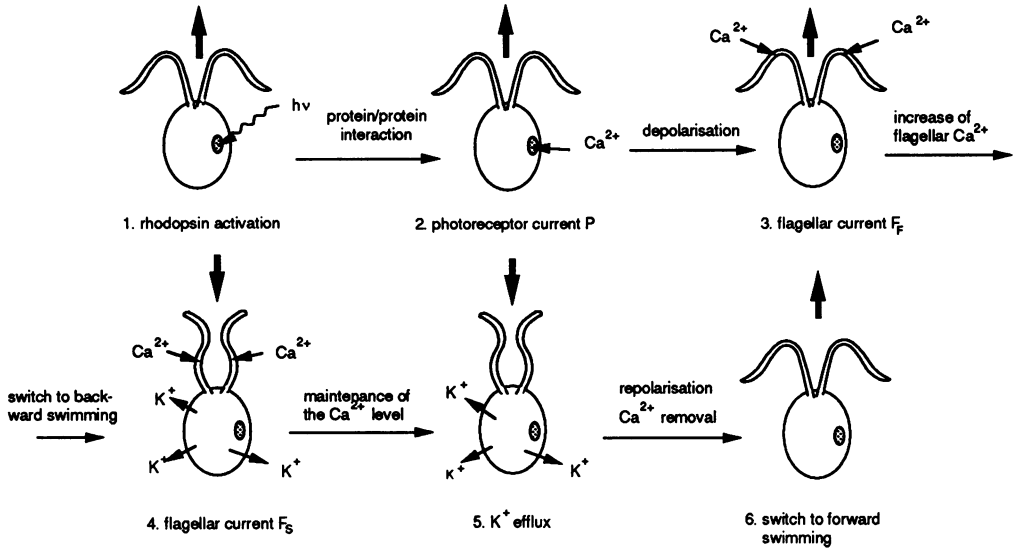

FIGURE 9 Current view of rhodopsin-regulated electrical events in *Chlamydomonas*. Six basic observations are plotted below the figures and the suggested processes above the arrows. Rhodopsin activates within 50 μs the photoreceptor channel P, most probably by a direct protein/protein interaction. If the P current depolarizes the cell below a critical level, a transient flagellar current F_F can be observed as an all-or-none event. Activation of delocalized K^+ channels (outward currents) facilitates prolonged Ca^{2+} inward currents into the flagella, which can be observed for some hundred milliseconds as a slow flagellar current F_S. The K^+ efflux seems to repolarize the cells close to the resting level before they resume foreward swimming.

trigger for voltage-gated flagellar inward currents. The transient current F_F is followed by a slow Ca^{2+} inward current F_S. Most of the Ca^{2+} that enters the flagellar over the whole length (Beck and Uhl, 1994) is bound to intraflagellar components. The long-lasting Ca^{2+} influx is accompanied by a simultanous K^+ outward current distributed over the whole plasmalemma. It is required for the control of the membrane potential during Ca^{2+} influx. Ca^{2+} down-regulates or partially blocks the channel and thereby guarantees a long-lasting but small Ca^{2+} influx. Both flagellar currents are involved in the photophobic response. F_F triggers the switch from foreward to backward swimming, whereas F_S keeps the cell in the backward-swimming mode for a prolonged time of 500 ms. The K^+ conductance can be used to finally repolarize the cell back to its resting state.

REFERENCES

Beck, C., and R. Uhl. 1994. On the localisation of voltage sensitive calcium channels in the flagella of *Chlamydomonas*. *J. Cell Biol.* 125: 1119–1125.

Deininger, W., P. Kröger, U. Hegemann, F. Lottspeich, and P. Hegemann. 1995. Chlamyrhodopsin represents a new type of sensory photoreceptor. *EMBO J.* 4:5849–5858.

Eckert, R., and P. Brehm. 1979. Ionic mechanisms of excitation in *Paramecium*. *Annu. Rev. Biophys. Bioeng.* 8:353–383.

Harz, H., and P. Hegemann. 1991. Rhodopsin regulated calcium currents in *Chlamydomonas*. *Nature.* 351:489–491.

Harz, H., C. Nonnengässer, and P. Hegemann. 1992. The photoreceptor current of the green alga *Chlamydomonas*. *Phil. Trans. R. Soc. Lond. B.* 338:39–52.

Holland, E.-M., F.-J. Braun, C. Nonnengässer, H. Harz, and P. Hegemann. 1996. The nature of rhodopsin triggered photocurrents in *Chlamydomonas*. I. Kinetics and influence of divalent ions. *Biophys. J.* 70:000–000.

Malhotra, B., and D. M. Glass. 1995. Potassium fluxes in *Chlamydomonas reinhardtii*. *Plant Physiol.* 108:1527–1536.

Sineshchekov, O. A. 1991. Photoreception in unicellular flagellates: bioelectric phenomena in phototaxis. *In* Light in Biology and Medicine, Vol. 2. R. H. Douglas, editor. Plenum Press, New York. 523–532.

Sineshchekov, O. A., F. F. Litvin, and L. Keszethely. 1990. Two components of the photoreceptor potential in phototaxis of the flagellated green alga *Haematococcus pluvialis*. *Biophys. J.* 57:33–39.

Smyth, R. D., J. Saranak, and K. W. Foster. 1988. Algal visual systems and their photoreceptor pigments. *Prog. Phycol. Res.* 6:255–285.