Properties of Tetraethylammonium Ion–resistant K^+ Channels in the Photoreceptor Membrane of the Giant Barnacle

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ABSTRACT   After the offset of illumination, barnacle photoreceptors undergo a large hyperpolarization that lasts seconds or minutes. We studied the mechanisms that generate this afterpotential by recording afterpotentials intracellularly from the medial photoreceptors of the giant barnacle *Balanus nubilus*. The afterpotential has two components with different time-courses: (*a*) an earlier component due to an increase in conductance to K^+ that is not blocked by extracellular tetraethylammonium ion (TEA^+) or 3-aminopyridine (3-AP) and (*b*) a later component that is sensitive to cardiac glycosides and that requires extracellular K^+, suggesting that it is due to an electrogenic Na^+ pump. The K^+ conductance component increases in amplitude with increasing Ca^{++} concentration and is inhibited by extracellular Co^{++}; the Co^{++} inhibition can be overcome by increasing the Ca^{++} concentration. Thus, the K^+ conductance component is Ca^{++} dependent. An afterpotential similar to that evoked by a brief flash of light is generated by depolarization with current in the dark and by eliciting Ca^{++} action potentials in the presence of TEA^+ in the soma, axon, or terminal regions of the photoreceptor. The action potential undershoot is generated by an increase in conductance to K^+ that is resistant to TEA^+ and 3-AP and inhibited by Co^{++}. The similarity in time-course and pharmacology of the hyperpolarizing afterpotentials elicited by (*a*) a brief flash of light, (*b*) depolarization with current, and (*c*) an action potential indicates that Ca^{++}-dependent K^+ channels throughout the photoreceptor membrane are responsible for all three hyperpolarizing events.

INTRODUCTION

The responses to light of a photoreceptor cell are shaped by a multitude of permeability and transport mechanisms in the cell membrane. Light-activated permeabilities (Millecchia and Mauro, 1969; Tomita, 1970; Brown et al., 1970; Brown and Mote, 1974) cause a change in receptor voltage, which in turn activates voltage-sensitive permeability mechanisms (Borsellino et al., 1965; Schwartz, 1976; Fain et al., 1977; Pepose and Lisman, 1978; Fain et al.,

1978; Bader et al., 1978). The flux of ions resulting from these changes can activate electrogenic pumps that provide a further influence on the membrane potential (Koike et al., 1971; Brown and Lisman, 1972). Synaptic interactions with other receptors or feedback from postsynaptic cells can also affect the amplitude and wave form of the visual signal (Baylor et al., 1971).

This paper is one of a series of reports on the conductances present in the photoreceptor neurons of the median eye of the giant barnacle, *Balanus nublius* Darwin (Hudspeth et al., 1977; Ross and Stuart, 1978; Edgington and Stuart, 1979). This 1-cm-long photoreceptor can be impaled with microelectrodes in its soma, or anywhere along the axon, including the region near the presynaptic terminals. In general, we have described the nature of conductances that influence the visual signal and their distribution along this decrementally conducting photoreceptor cell. In the present study, we describe K^+ channels in the photoreceptor membrane that are sensitive to the external Ca^{++} concentration. Ca^{++}-sensitive K^+ channels have been reported in the somata of the lateral photoreceptors of *Balanus eburneus* (Hanani and Shaw, 1977). We show here that the Ca^{++}-dependent K^+ channels in *B. nubilus* are present all along the receptor cell, including the presynaptic terminal region. Conductances such as this one, which affect the rate of repolarization of the receptor at the offset of illumination, are of particular interest because higher order cells in the barnacle visual system respond to the dimming rather than the brightening of light (Gwilliam, 1963; Stuart and Oertel, 1978) and are sensitive to the rate of repolarization of the receptor (Ozawa et al., 1976).

METHODS

Experiments were carried out on medial photoreceptors of the giant barnacle (*Balanus nubilus* Darwin). Previous papers provide details of the dissections and methods for recording and stimulating with current and light (Hudspeth and Stuart, 1977; Hudspeth et al., 1977; Ross and Stuart, 1978). Our use of somatic, axonal, and terminal "regions" of the receptor follows the convention presented in Edgington and Stuart (1979).

Photoreceptors were impaled under visual control with a microelectrode to record membrane potential, and in some experiments were also impaled with a second microelectrode near the first to inject current, as previously described (Edgington and Stuart, 1979). The normal physiological saline contained 461.5 mM NaCl, 8 mM KCl, 20 mM CaCl₂, 12 mM MgCl₂, and 10 mM Tris-HCl buffer adjusted to pH 7.7 (Brown et al., 1970). Solutions containing tetraethylammonium chloride (TEA; Eastman Organic Chemicals Div., Eastman Kodak, Rochester, N. Y.), 3-aminopyridine (3-AP, Sigma Chemical Co., St. Louis, Mo.) and altered Ca^{++} concentration were made as described elsewhere (Edgington and Stuart, 1979). In solutions with altered K^+ concentration, K^+ was replaced with Na^+. CoCl₂ was added to experimental solutions. Cl^--free saline contained sodium methylsulfate, potassium methylsulfate, calcium ethylsulfate, and magnesium ethylsulfate (City Chemical Corp., New York) substituted for the Cl^- salts, with the pH adjusted to 7.7 with 1 M NaOH; tetraethylammonium acetate (Eastman) was added to Cl^--free saline. In Cl^--free saline the Ag-AgCl reference electrode developed a liquid-junction potential of about +90 mV, so a potassium acetate microelectrode connected to an electrometer served as the reference electrode.

Ouabain and strophanthidin (Sigma) were dissolved in normal saline by vigorous stirring; dihydrodigitoxigenin (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) was dissolved by vigorous stirring at 4°C for at least 24 h. Experiments were conducted at room temperature (18°–22°C).

RESULTS

Generation of the Hyperpolarizing Afterpotential by a TEA⁺-resistant K⁺ Conductance and a Na⁺ Pump

Fig. 1 illustrates the response of a median photoreceptor to pulses of light of increasing duration. The receptor depolarizes in response to light and at the offset of the light hyperpolarizes beyond the dark resting potential. As in barnacle lateral photoreceptors (Koike et al., 1971), the duration of the hyperpolarizing afterpotential grows with increasing duration of the light pulse.
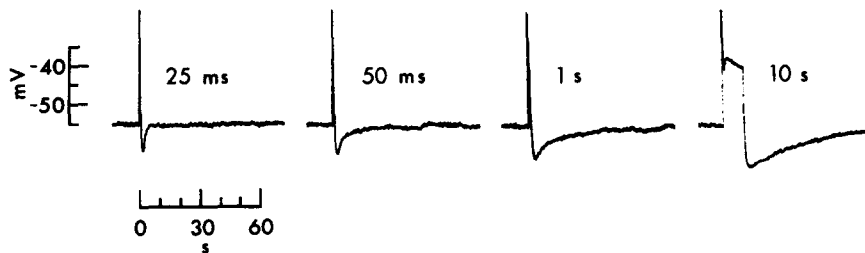

FIGURE 1.   Responses to steps of light of various durations recorded from a photoreceptor soma. The amplitude and duration of the hyperpolarizing afterpotential increase with increasing duration of illumination. The duration of the light stimulus of $10^{-2.0}$ mW/cm² intensity is indicated above each trace. Dark resting potential was −55 mV.

At certain combinations of light intensity and duration it was apparent that the afterpotential of the light response decayed in a complex manner. Fig. 2 *A* and *B* shows recordings from two receptors that displayed a two-component hyperpolarization. In normal (8 mM K⁺) saline, at the end of a step of light, each receptor hyperpolarized rapidly; the membrane potential then returned to its dark resting value at first relatively quickly, then more slowly, over a period of 1–2 min. The experiments described below show that the early phase of the hyperpolarization is due to a long-lasting increase in conductance to K⁺ and suggest that the slower phase is due to an electrogenic Na⁺ pump.

EFFECT OF ALTERED K⁺ CONCENTRATION ON THE HYPERPOLARIZING AFTER-POTENTIAL   Removing extracellular K⁺ (Fig. 2 *A*) or doubling its concentration (Fig. 2 *B*) markedly affected the afterpotential. In K⁺-free saline, the cell depolarized in the dark, the early component of the hyperpolarizing afterpotential was enhanced, and the late component was abolished. In 16 mM K⁺ the dark resting potential did not change, the early phase of the afterpotential was smaller, and the late component was unaffected.

These results suggest that immediately after the light is turned off the membrane potential of the photoreceptor is dominated by a high conductance to K^+. Since in K^+-free saline the K^+ equilibrium potential would be more negative, and in 16 mM K^+ more positive, than in normal (8 mM K^+) saline, the membrane potential immediately after the light pulse would vary accordingly. On the other hand, the late phase of the hyperpolarization is abolished by the K^+-free saline but unaffected by increasing the K^+ concentration. This result suggests that during this late period the membrane potential is domi-
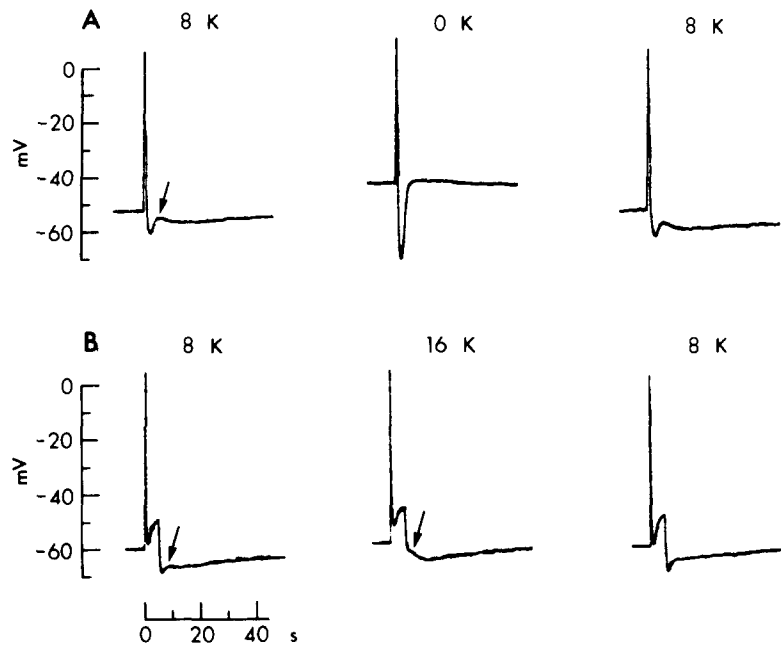

FIGURE 2.   Effect of changes in extracellular K^+ concentration on the hyperpolarizing afterpotential of the light response. Responses to (A) 1-s and (B) 5-s steps of light of $10^{-0.8}$ mW/cm^2 intensity recorded in two photoreceptor somata. The K^+ millimolar concentration of the saline bathing the receptor is indicated above each record. The arrows point to the beginning of the slow hyperpolarization that interrupted the decay of the hyperpolarizing afterpotential (see text).

nated by an electrogenic Na^+ pump, since such pumps in other nerve cells have an absolute requirement for extracellular K^+. An explanation for the depolarization of the cell in K^+-free saline would be provided if it were found that an electrogenic Na^+ pump contributes to the dark resting potential of this cell.

An alternative possibility is that the late component of the afterpotential disappears in K^+-free saline as a consequence of the cell's depolarization. The experiment of Fig. 3 shows that depolarization in fact cannot account for the late component's disappearance. Removal of K^+ had little effect on the membrane potential of some receptors, two of which are shown in Fig. 3 A

and *B*. Bathing a receptor in K^+-free saline in the dark caused only a 2-mV change in its resting potential (Fig. 3 *A*). Return of normal saline caused a hyperpolarization of 4 mV that slowly decayed to resting potential. Another receptor (Fig. 3 *B*) was bathed first in normal, then in K^+-free saline, while steps of light were delivered to it from dark. At the end of a step of light in
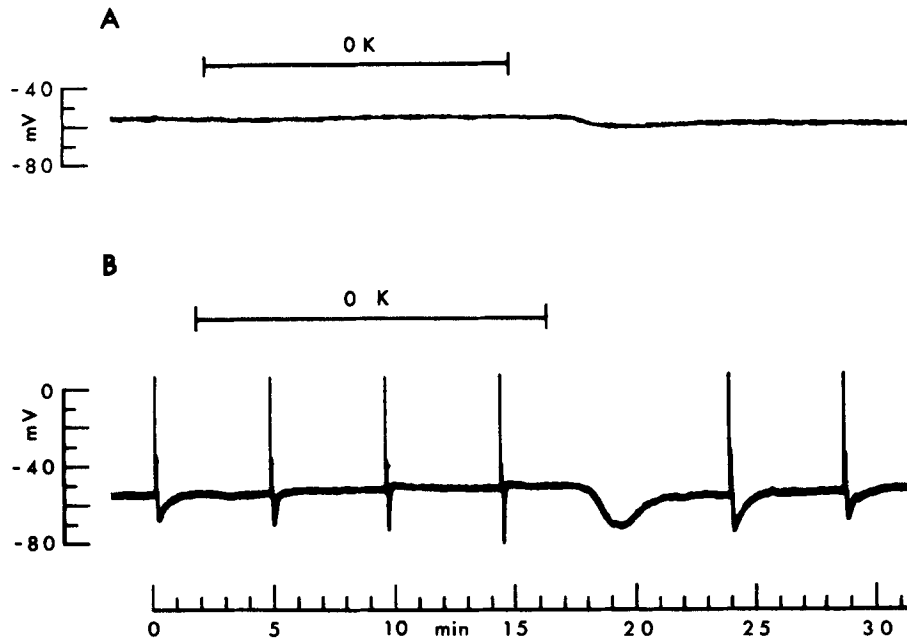

FIGURE 3.   Effect of K^+ removal and return on the dark resting potential. (*A*) Bathing a photoreceptor in K^+-free saline in the dark caused a 2-mV depolarization. On return of normal saline to the bath, the receptor hyperpolarized transiently. (*B*) 5-s steps of $10^{-0.8}$ mW/cm² light were delivered every 4.7 min while a receptor was bathed in K^+-free saline. On return of normal saline to the bath, the receptor hyperpolarized. The hyperpolarization produced by the reintroduction of K^+ was much larger when steps of light were delivered during K^+-free superfusion than when the cell was kept in the dark in K^+-free saline. The delay between introduction of normal saline and the hyperpolarization was due to a delay in the perfusion system. Receptors in *A* and *B* were from different preparations. In repetitions of this experiment, in which the same photoreceptor was tested in situations *A* and *B* with protracted (15 or more min) and identical times of exposure to K^+-free saline, the same results were obtained.

normal K^+ the receptor displayed a hyperpolarizing afterpotential. In K^+-free saline there was little change in the dark resting potential or in the depolarization caused by light, but the hyperpolarizing afterpotential became much larger and briefer. On return to normal saline the cell underwent a large (25-mV), long-lasting hyperpolarization in the dark that decayed slowly (over

several minutes). The hyperpolarizing afterpotential of the light response regained its late decay phase upon return of K^+ to the saline.

A similar large hyperpolarization has been observed in other cells with electrogenic pumps after the return of K^+ to the extracellular fluid after these cells have been fired tetanically in K^+-free saline (Rang and Ritchie, 1968; Baylor and Nicholls, 1969), or after the cells have been injected with Na^+ while bathed in K^+-free saline (Thomas, 1969; Brown and Lisman, 1972). The interpretation of these results can be applied to those illustrated in Fig. 3: in K^+-free saline, the internal Na^+ concentration was raised during successive steps of light, and when K^+ was returned to the external solution the Na^+ was extruded electrogenically, causing a large hyperpolarization.

It was also possible to separate the early and late components of the afterpotential by treatment with cardiac glycosides. Ouabain and strophanthidin affect electrogenic Na^+ pumps in a variety of cells with a high degree of specificity but their effects are usually irreversible; dihydrodigitoxigenin specifically and reversibly inhibits electrogenic Na^+ pumping in squid giant axon (J. G. Perry and P. De Weer, unpublished data; see Abercrombie and De Weer [1978]). 10^{-5} M dihydrodigitoxigenin reversibly blocked the late component of the afterpotential, but did not affect the early component. Ouabain and strophanthidin had effects similar to dihydrodigitoxigenin, but, except for brief exposure of 3 min or less, the effects were irreversible. Altogether, these results provide evidence that an electrogenic Na^+ pump contributes to the hyperpolarizing afterpotential of the light response, as it does in lateral receptors of *B. eburneus* (Koike et al., 1971) but that it is responsible only for the late component.

The results presented above show that in zero K^+ one observes only the early component of the afterpotential. Another way of observing only this component was suggested by the observations presented in Fig. 1: as the duration of the light stimulus was shortened, the afterpotential also became shorter. A sufficiently brief flash might activate the early component but not cause enough Na^+ entry to activate a pump substantially. Fig. 4 A shows that the amplitude of the hyperpolarization after a brief flash of light depended on external K^+ and that the duration of this event was similar in 0 and 8 mM K^+. The similarity of the duration in 0 K^+, when the later component was blocked, and in the presence of K^+ suggest that a pump contributed relatively little to the hyperpolarization after a brief flash. Thus, brief flashes were used to study further the properties of the conductance increase underlying the early part of the afterpotential.

The postflash afterpotential showed a reversal potential (Fig. 4 B). Although, due to the slowness of the afterpotential, the exact value of this reversal potential was sometimes difficult to determine, it clearly depended on extracellular K^+ concentration. In 16 mM K^+ (Fig. 4 B), the afterpotential reversed sign at about -60 mV; in 2 mM K^+ it reversed at about -100 mV. Thus, for an eightfold decrease in K^+ concentration, the reversal potential changed by ~40 mV. This value is less than that predicted for a K^+ electrode (52 mV, i.e., 58 × log 8), but it is possible that the light-activated Na^+

conductance decays with a slow time-course, like that of the K$^+$ conductance, after termination of a bright light (Baumann and Hadjilazaro, 1972; Brown and Cornwall, 1975), and thus contributes to the reversal potential.

Tetraethylammonium ion (TEA$^+$) and 3-aminopyridine (3-AP), agents that specifically block K$^+$ channels, were tested to determine whether they would block the K$^+$ component of the afterpotential. The afterpotential after either a flash or a step of light was not diminished by bathing the receptor in 30 mM 3-AP or 100 mM TEA$^+$, the highest concentrations tested. Consequently, the K$^+$ channels responsible for the early component of the afterpotential will be referred to as "TEA$^+$-resistant K$^+$ channels."

Evidence for Activation of the TEA$^+$-resistant K$^+$ Channels by Ca^{++}

The TEA$^+$-resistant increase in K$^+$ conductance might be activated by the light itself, by ion entry activated by light, or as a result of the depolarization
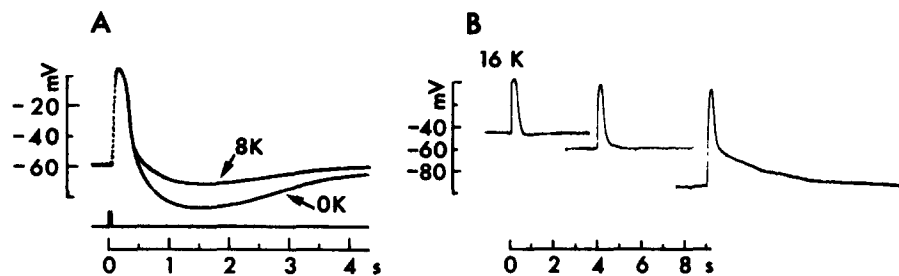

FIGURE 4. (*A*) Responses to brief flashes of light in 0 and 8 mM K$^+$ recorded in a receptor soma. The 25-ms flash of light was of $10^{-0.5}$ mW/cm^2 intensity. (*B*) Reversal potential in 16 mM K$^+$ of the hyperpolarizing afterpotential. Responses to 50-ms flashes of light of $10^{-1.1}$ mW/cm^2 intensity recorded in a receptor axon 1.0 mm from the soma. The receptor was polarized to various membrane potentials by passing steady current through a nearby second microelectrode. The reversal potential was about −60 mV. Receptors in *A* and *B* were from different preparations.

caused by light. In Fig. 5 the hyperpolarization after a flash of light (Fig. 5 *A*) is compared with that after a brief pulse of depolarizing current (Fig. 5 *B*) injected into a photoreceptor. The cell was bathed in K$^+$-free saline to block possible contributions from a Na$^+$ pump. The afterpotential after the break of the current pulse is similar in amplitude and duration to the hyperpolarizing afterpotential elicited by light. In other experiments it was shown that afterpotentials after depolarizing currents were not blocked by external TEA$^+$. It is likely, then, that the increase in K$^+$ conductance after a flash is not due to light acting directly on a K$^+$ conductance.

Ca^{++}-SENSITIVITY OF THE AFTERPOTENTIAL Experiments were carried out to determine whether the TEA$^+$-resistant increase in conductance to K$^+$ was caused directly by depolarization itself or indirectly by the influx of ions during the depolarization. In a variety of cells, K$^+$ conductances activated by

Ca^{++} have been demonstrated (see review by Meech [1976]). Such channels are often relatively insensitive to TEA^+ (Neher and Lux, 1972; Barrett and Barrett, 1976; Thompson, 1977). It is reasonable that a Ca^{++}-activated K^+ conductance might be present in the photoreceptor because (a) intracellular Ca^{++} increases during illumination in barnacle photoreceptors (Brown and Blinks, 1974) and (b) Ca^{++} action potentials followed by long-lasting, TEA^+-resistant undershoots can be elicited from the photoreceptor (Ross and Stuart, 1978).

Fig. 6 shows the effect of external Ca^{++} in various concentrations on the afterpotential after a flash of bright light. As the Ca^{++} concentration was reduced from 32 to 8 mM, the afterpotential became smaller and shorter. No
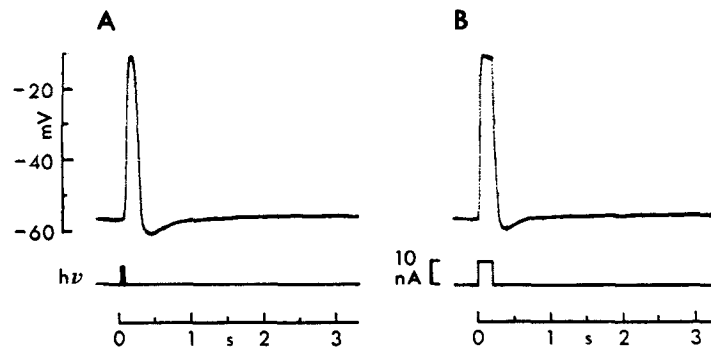

FIGURE 5. Comparison of the hyperpolarizing afterpotential elicited by (A) a flash of light and (B) a brief depolarizing current pulse. A photoreceptor axon was impaled with two microelectrodes placed 1.0 and 1.3 mm from the soma. A shows the response to a 50-ms flash of light of $10^{-2.0}$ mW/cm^2 intensity. B shows a brief depolarizing current pulse that elicited a depolarization as large as the depolarization caused by light. On the break of the current pulse, the receptor hyperpolarized. The hyperpolarizing afterpotential elicited by current had a time-course similar to that evoked by light, but it was slightly smaller in amplitude and of shorter duration. The experiment was carried out in K^+-free saline.

hyperpolarizing afterpotential was generated in 4 mM Ca^{++}. The peak amplitude of the depolarization caused by light was little affected by changes in Ca^{++} concentration; however, the depolarization fell from its peak value more slowly as the Ca^{++} concentration was reduced.

If the afterpotential is a consequence of the entry of Ca^{++} into the cell, the addition of Co^{++} to the saline would be expected to decrease the amplitude and duration of this event; Co^{++} generally inhibits Ca^{++} currents. Fig. 7 A shows the effect of extracellular Co^{++} in various concentrations on the receptor of Fig. 6. As the Co^{++} concentration was increased, the hyperpolarizing afterpotential became smaller and briefer. The receptor also depolarized in the Co^{++} solutions, but the peak amplitude of the depolarization caused by

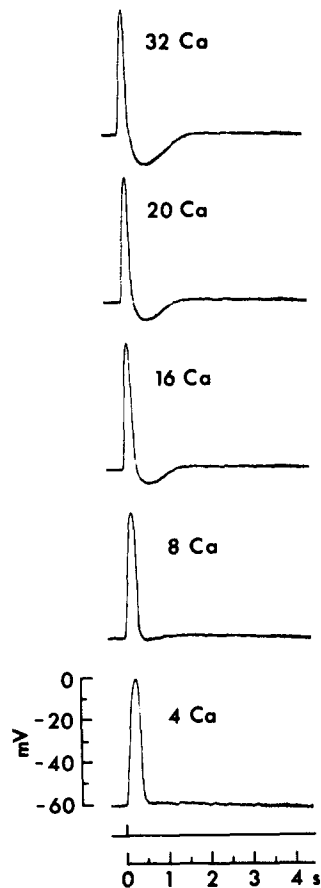FIGURE 6.   Dependence on external Ca^{++} of the amplitude and duration of the hyperpolarizing afterpotential of the response to a flash of light. Shown are responses, recorded in the receptor axon hillock (550 μm from the soma), to 20-ms flashes of $10^{-1.7}$ mW/cm^2 light in various millimolar Ca^{++} concentrations, which are indicated to the right of each trace. The hyperpolarizing afterpotential was not elicited in 4 mM Ca. The amplitude and duration of the afterpotential increased as the Ca^{++} concentration increased. Ca^{++} replaced Mg. The preparation was mounted in a two-compartment chamber. The experimental solutions bathed the soma and initial 3.2 mm of axon; the rest of the receptor was superfused with normal saline throughout. Dark resting potential in normal (20 mM Ca) saline was −58 mV; in 4 and 8 mM Ca, −61 mV; in 16 mM Ca, −58 mV; and in 32 mM Ca, −59 mV.

light was little affected. 30 mM Co^{++} blocked the afterpotential. The effects of Co^{++} were reversible. Fig. 7 *B* shows that the addition of Ca^{++} to the 6 mM Co^{++} solution partially overcame the inhibition of the afterpotential by Co^{++}; this result shows that the effect of Co^{++} on the afterpotential is specific and not due to "screening" of negative surface charges.

The afterpotentials displayed a different wave form in Co^{++} than in normal saline: the residual hyperpolarizing afterpotential was followed by a rebound depolarization that slowly decayed to rest (Fig. 7 A). A similar rebound depolarization was observed in K^+-free saline (Figs. 2 and 3) and dihydrodigitoxigenin, and on occasion in normal saline. It is likely that the depolarizing phase of the afterpotential is due to a long-lasting, light-activated conductance, since it is not observed after depolarization by current (Fig. 9 B, right); this depolarization may normally be masked by the hyperpolarizing afterpotential.
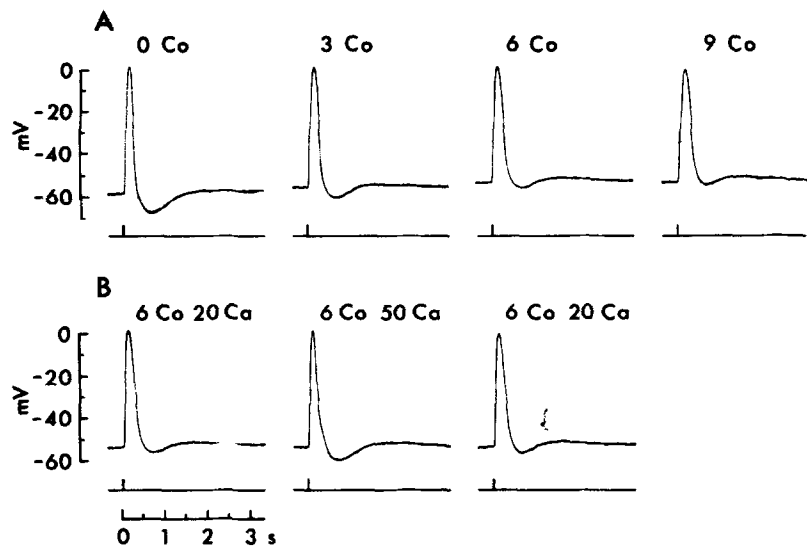

FIGURE 7.   Effect of Co^{++} on the hyperpolarizing afterpotential of the response to a flash of light. (A) Responses to a flash of light in normal saline (left trace) and in increasing concentrations of Co^{++} added to normal saline. The millimolar Co^{++} concentration is indicated above each trace. The hyperpolarizing afterpotential decreased in amplitude and duration as the Co^{++} concentration was increased. (B) The inhibition of the afterpotential by 6 mM Co^{++} can be partially overcome by increasing the Ca^{++} concentration to 50 mM (center trace). This figure shows the same cell, site of impalement, and superperfusion arrangement as Fig. 6. Elevated Ca^{++} saline was made by adding $CaCl_2$ to the 6 mM Co^{++} solution. The 20-ms flashes of light were of $10^{-1.7}$ mW/cm^2 intensity.

Thus, the amplitude of the hyperpolarizing afterpotential after the response to a flash of light depends upon external Ca^{++} concentration. The observation that the afterpotential is blocked by Co^{++} or low Ca^{++} indicates that Ca^{++} activates these K^+ channels and that they are not directly activated by voltage.

TEA^+-RESISTANT K^+ CONDUCTANCE GENERATES THE UNDERSHOOT OF Ca^{++}

ACTION POTENTIALS   In normal saline, the photoreceptor does not show regenerative activity. In the presence of low (millimolar) concentrations of TEA^+, or in 3-AP, a Ca^{++}-dependent action potential can be generated upon depolarization with light or current (Ross and Stuart, 1978; Edgington and

Stuart, 1979). This action potential is followed by a long-lasting undershoot that persists even in 400 mM TEA$^+$ (Ross and Stuart, 1978). The action potential and its undershoot can be elicited from the soma, the axon, and the presynaptic terminal region of the cell (Edgington and Stuart, 1979). The experiments described below show that the undershoot of the Ca^{++} action potential is due to TEA$^+$-resistant, Ca^{++}-dependent K$^+$ channels in the receptor membrane, most likely the same channels that are activated by depolarization with light or current.

The undershoot of the action potential is due to an increase in conductance whose reversal potential depends on extracellular K$^+$. Fig. 8 shows reversal of undershoots of current-evoked action potentials set up in the axon (Fig. 8 *A*) and in the terminal region (Fig. 8 *B*) of photoreceptors bathed in TEA$^+$. The undershoots went through a null and then reversed sign as the membrane was hyperpolarized with injected current. The values of the reversal potentials varied with extracellular K$^+$ concentration (*graphs* in Fig. 8). The reversal potentials for action potentials set up in the axons of the two photoreceptors tested could be fitted with a line of slope 58 mV/10-fold change in K$^+$ concentration. This relationship is predicted from the Nernst equation for a pure K$^+$ event. Thus, the undershoot of the action potential in the axon is due solely to an increase in conductance to K$^+$.

The reversal potentials of action potentials elicited in the terminal region of three photoreceptors, however, described lines of 55 (Fig. 8 *B*), 49, and 40 mV per 10-fold change in K$^+$ concentration. In the terminal region, some factor besides the increased conductance to K$^+$ appears to contribute to the amplitude of the undershoot. One possibility is that there is an increase in Cl$^-$ conductance during the undershoot. However, replacement of all external Cl$^-$ with methylsulfate and ethylsulfate had no effect on the amplitude or reversal potential of the undershoot. Thus, it is unlikely that a Cl$^-$ conductance contributes to the action potential undershoot. The failure of the action potential undershoot in the terminal region to follow K$^+$ concentration with a Nernst slope remains unexplained.

We conclude that, in both the axon and the terminal region, a slow TEA$^+$-resistant increase in K$^+$ conductance follows the regenerative entry of Ca^{++}. It seemed likely that the Ca^{++} entry activates the same kind of TEA$^+$-resistant K$^+$ channels that are activated during illumination. It had been shown that the amplitude of both the action potential and its undershoot depends on external Ca^{++} (Ross and Stuart, 1978), but from this observation one could not distinguish whether the undershoot depends on the voltage change or on Ca^{++} entry during the action potential. We tested the hypothesis that the undershoot depends on Ca^{++} entry by making use of Co^{++}.

Fig. 9 *A* shows that Co^{++} blocks both the action potential and the increase in conductance to K$^+$. A brief depolarizing current pulse, delivered to a photoreceptor bathed in 10 mM TEA$^+$, elicited an action potential that was followed by a large, long-lasting undershoot. The addition of 30 mM Co^{++} to the TEA$^+$ solution blocked the regenerative response to depolarization. A current pulse that depolarized the membrane to a potential even larger than

the peak amplitude of the action potential was followed by a simple repolarization to the resting potential. This result suggests that the undershoot is not a result of the depolarization produced by the action potential but is a consequence of the Ca^{++} current that underlies the action potential.
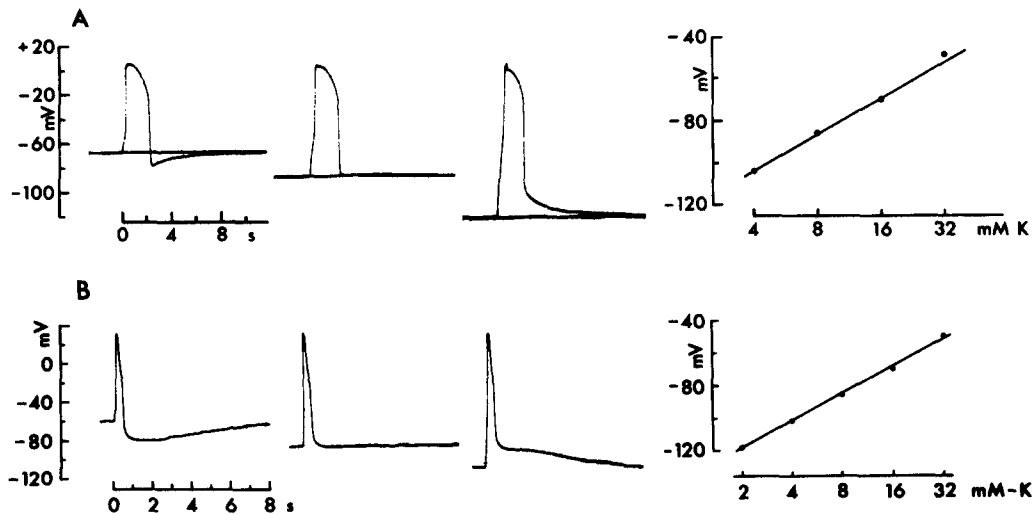

FIGURE 8. K^+ dependence of the reversal potential of action potential undershoots. Each action potential was elicited in (A) the axon or (B) the terminal region of a photoreceptor with a brief depolarizing current pulse from a nearby microelectrode. Traces in A and B are taken from two different preparations. To the right of each set of traces, the reversal potentials of the undershoots are plotted against K^+ concentration on a log scale. (A) Impaled axonal region bathed in 50 mM TEA^+ and 8 mM K^+; somatic and terminal regions bathed in 20 mM Co^{++}. The undershoot reversed sign when the cell was polarized to −84 mV. The points on the graph are measured reversal potentials; the line has a slope of 58 mV/10-fold change in K^+ concentration. (B) Receptor impaled 320 μm proximal to the commissure of the ganglion (i.e., ∼500 μm from the presynaptic arborization). Terminal region bathed in 50 mM TEA^+ and 8 mM K^+; somatic and axonal regions bathed in 20 mM Co^{++}. The undershoot reversed sign at −86 mV. The line on the graph drawn through measured reversal potentials has a slope of 55 mV/10-fold change in K^+ concentration. In each experiment the compartment containing TEA^+ was also low in Na^+; Na^+ was replaced by tetramethylammonium ion (TMA^+); TEA^+ plus TMA^+ equalled 400 mM. The estimated measurement error for reversal potentials is ±2 mV.

In normal saline (Fig. 9 B), the membrane displayed rectification of slow onset in response to depolarizing current. On the break of a depolarizing current pulse, the membrane hyperpolarized. Bathing the terminal in 30 mM Co^{++} blocked the slow rectification and the hyperpolarization. Thus, this rectification is caused by Ca^{++} entry during depolarization, which in turn causes an increase in conductance to K^+.
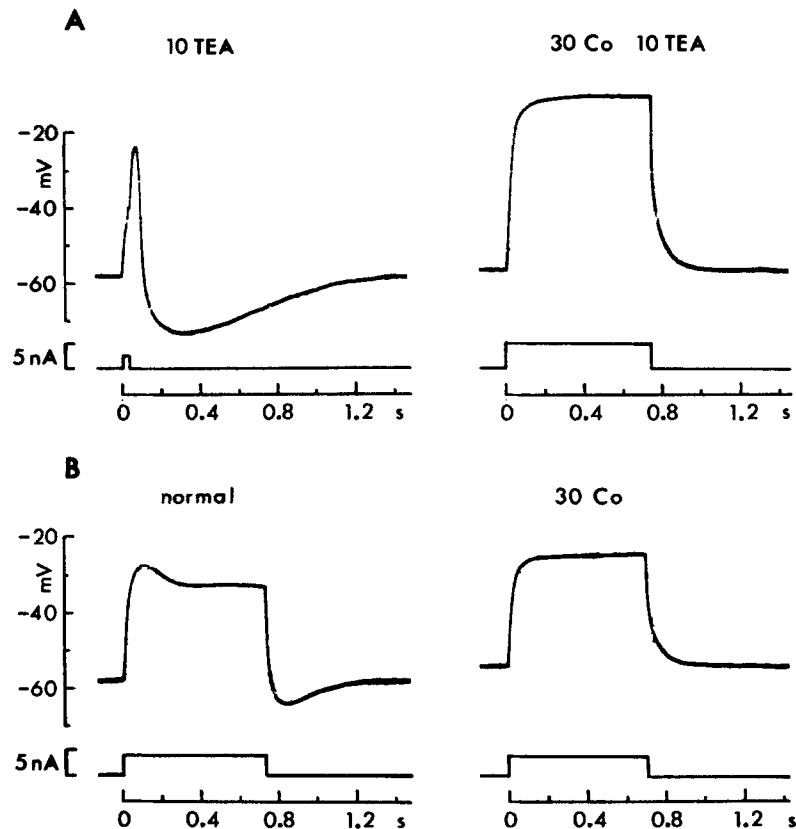
FIGURE 9.   The effect of Co^{++} on (A) the Ca^{++} action potential in a photoreceptor and (B) rectification. A photoreceptor axon was impaled near the terminal region with two microelectrodes (960 and 1,050 μm from the commissure). (A) When the terminal region of the receptor cell was bathed in 10 mM TEA^+, an action potential followed by an undershoot was elicited with a brief depolarizing current pulse. The threshold depolarization was 14 mV. When 30 mM Co^{++} was added to the TEA^+ solution, depolarization with current produced an electrotonic response that was followed by a simple repolarization. The effects of Co^{++} were reversible. (B) A depolarizing current pulse in normal saline resulted in a depolarization that fell from its peak value to a maintained plateau (rectification). The break of current was followed by a hyperpolarizing afterpotential. The addition of 30 mM Co^{++} reversibly blocked the rectification and afterpotential. TEA^+ replaced Na^+. The preparation was situated in a two-compartment bath as in Fig. 10.

It was possible to overcome the Co^{++} inhibition of the undershoot by raising the Ca^{++} concentration (Fig. 10). In 5 mM Ca^{++}, it was possible to elicit a relatively small action potential followed by an undershoot with a brief current pulse (Fig. 10 A). The addition of 5 mM Co^{++} inhibited the regenerative current; however, we were able to depolarize the membrane with current (Fig. 10 B) to a level more positive than that reached by the peak of the

action potential in Fig. 10 *A*. The amplitude and duration of the undershoot after the break of this depolarizing current was substantially decreased in Co^{++}. That the undershoot was not entirely abolished indicates that the Co^{++} inhibition of the Ca^{++} current during depolarization was not complete at these concentrations. When the Ca^{++} concentration in the Co^{++} solution was increased to 25 mM (Fig. 10 *C*), the action potential was restored and was followed by an undershoot that was larger and longer than that produced by depolarization in low Ca^{++} plus Co^{++} (Fig. 10 *B*). This result rules out an effect of Co^{++} on the undershoot mechanism itself (for example, through
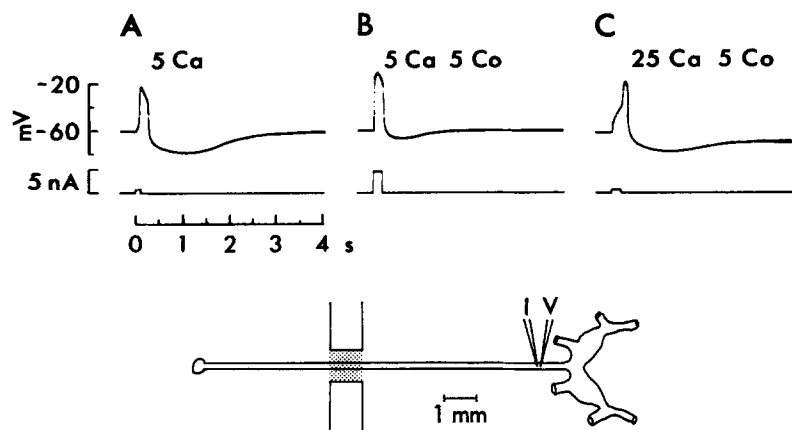

FIGURE 10. Co^{++} inhibition of the action potential and undershoot are overcome with added Ca^{++}. A photoreceptor was impaled in the terminal region (drawing). (*A*) An action potential elicited in 5 mM Ca^{++} and 100 mM TEA^+ by a brief current pulse shows a large undershoot. (*B*) When 5 mM Co^{++} was added to the 5 mM Ca^{++} saline, no action potential was elicited. The membrane was depolarized with a current pulse to a level greater than that achieved by the action potential in *A*, yet the hyperpolarization after this event was small. (*C*) When the Ca^{++} concentration in the Co^{++} solution was raised to 25 mM, the action potential and its undershoot were restored. Ca^{++} was replaced by sucrose; TEA^+ replaced Na^+.

screening of surface charges on the membrane) and supports the notion that the action of Co^{++} is to inhibit Ca^{++} current.

The experiments presented in Figs. 8, 9, and 10 provide evidence that the conductance responsible for the action potential undershoot is a K^+ conductance dependent upon Ca^{++} entry. Because action potentials elicited in all regions of the cell are followed by undershoots (Edgington and Stuart, 1977 and 1979), these Ca^{++}-activated K^+ channels must be present throughout the receptor membrane.

DISCUSSION

The primary response to light of barnacle photoreceptors is an increase in conductance to Na^+ (Brown et al., 1970) and a resulting inward Na^+ current.

Ca^{++} also enters the cell, and the intracellular Ca^{++} concentration increases (Brown et al., 1970; Brown and Blinks, 1974). Both the Na$^+$ and Ca^{++} currents tend in themselves to depolarize the cell. This paper describes two mechanisms, activated by the entry of the Na$^+$ and Ca^{++} during light, which exert an opposite, hyperpolarizing force on the membrane potential.

One mechanism has an absolute requirement for K$^+$ and is sensitive to cardiac glycosides. Thus, it has characteristics of the electrogenic Na$^+$ pump seen in receptors of *Limulus* (Brown and Lisman, 1972) and of barnacle lateral eye (Koike et al., 1971). This pump can drive the membrane potential of barnacle medial receptors to values as great as −120 mV after the offset of light (Hudspeth and Stuart, 1977), and its effect on the membrane potential decays relatively slowly. By analogy with other preparations, the function of the pump would be to extrude the Na$^+$ that enters the receptor during the light.

The second mechanism is an increase in conductance to K$^+$. This K$^+$ channel is sensitive to the external Ca^{++} concentration, is blocked by external Co^{++}, and is insensitive to external TEA$^+$. The afterhyperpolarization caused by this K$^+$ conductance increase lasts hundreds of milliseconds or seconds. We suggest that this K$^+$ conductance is activated by the entry of Ca^{++} that results from the depolarization of the receptor by light.

The effect on membrane potential of these two mechanisms is most obvious after the sudden offset of illumination. However, both the conductance to K$^+$ and the activity of the pump presumably are increased with respect to the dark resting level during maintained illumination. Evidence for an increase in a Ca^{++}-dependent K$^+$ conductance immediately after the onset of light has been presented by Hanani and Shaw (1977).

TWO TYPES OF K$^+$ CHANNELS IN THE PHOTORECEPTOR MEMBRANE   The evidence now suggests that there are two types of K$^+$ channel in the photoreceptor membrane, one sensitive to and the other resistant to external TEA$^+$. We infer that a K$^+$ channel sensitive to TEA$^+$ is present because bathing the receptor in TEA$^+$ or in 3-AP permits us to elicit Ca^{++} action potentials from the cell (Ross and Stuart, 1978). As the concentration of external TEA$^+$ is increased from 1 to 10 mM the threshold of the action potential decreases, but above 10 mM it does not change, indicating that the channel is sensitive to TEA$^+$ in this range (Ross and Stuart, 1978). The second K$^+$ channel, described in the present paper, is responsible for the undershoot of the Ca^{++} action potential. This undershoot persists even in 400 mM TEA$^+$. Other neuronal types have been described that have several K$^+$ conductances that can be distinguished from one another by their different sensitivities to TEA$^+$ and the aminopyridines (Neher and Lux, 1972; Barrett and Barrett, 1976; Thompson, 1977); in these cases, it is the Ca^{++}-dependent K$^+$ conductance that is least sensitive to external TEA$^+$ and that has the slowest time-course of the conductances. However, S. Bolsover and R. W. Meech (personal communication) have found in *B. eburneus* photoreceptors a slowly developing outward current that has the characteristics of a Ca^{++}-dependent K$^+$ current that is inhibited by TEA$^+$ (50 mM) applied externally.

FUNCTIONAL SIGNIFICANCE OF THE TEA^+-RESISTANT K^+ CHANNELS The experiments presented in Figs. 6 and 7 show that factors that affect the TEA^+-resistant K^+ conductance also affect the rate of repolarization of the receptor membrane at the offset of light. The rate of repolarization of the cell after a flash of light was less in Co^{++} or in low-Ca^{++} salines, where the afterhyperpolarization was also diminished.

The changes in membrane potential upon the offset or dimming of light are of particular interest when considering the processing of information by higher-order neurons. It is the dimming of light that elicits a behavioral response in the animal (Gwilliam, 1963) and that leads to responses in higher-order neurons of the visual pathway (Gwilliam, 1963; Ozawa, et al., 1976; Stuart and Oertel, 1978). The amplitude of the excitatory postsynaptic potential in third-order cells of the pathway (Stuart and Oertel, 1978) increases with the rate of fall of photoreceptor voltage (Ozawa et al., 1976). For transmission of information to take place in the pathway, it is imperative that the receptor voltage fall relatively rapidly upon dimming at a rate no slower than 5 mV/s (Oertel and Stuart, unpublished observations); however, light-activated channels are thought to turn off slowly relative to this value (Baumann and Hadjilazaro, 1972). Thus, it is reasonable that a functional role of the TEA^+-resistant K^+ current is to enhance the rate of fall of the membrane potential upon the dimming of light.

The TEA^+-resistant K^+ channels may also participate in the control of the membrane potential in the receptor axon and presynaptic terminals. The Ca^{++} current entering the cell along its length in response to depolarization is normally prevented from becoming regenerative by a countercurrent through the TEA^+-sensitive and TEA^+-resistant channels. Consequently, the potential changes in response to light are normally graded all along the axon. Although the TEA^+-resistant K^+ current alone is insufficient to control the receptor voltage, since action potentials are generated when the TEA^+-sensitive channels are inhibited, if the TEA^+-resistant K^+ channels are Ca^{++}-activated, as suggested, they could act as a negative-feedback mechanism to control the amount of Ca^{++} entering the axonal and terminal regions upon depolarization.

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