Hyperpolarization of a Barnacle Photoreceptor Membrane following Illumination

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ABSTRACT Membrane potential changes following illumination of a photoreceptor cell in the lateral ocellus of a barnacle (*Balanus eburneus*) were studied by means of intracellular recording and polarization techniques. Illumination produces a depolarizing response. When the illumination is terminated, the membrane potential temporarily becomes more negative than the resting potential prior to illumination. Although the amplitude of this postillumination hyperpolarization depends upon the intensity as well as the duration of the light pulse, the time course is fairly constant. The hyperpolarization is not associated with any significant membrane conductance increase and is abolished by 10^{-5} M ouabain. It diminishes when the external Na or K ions are removed. An intracellular injection of Na ions produces a hyperpolarization similar to that following illumination. It is suggested that the postillumination hyperpolarization is produced by an electrogenic Na pump which is activated by the Na influx during illumination.

INTRODUCTION

Illumination of the barnacle photoreceptor produces a depolarization of the membrane, the result of a conductance increase mainly to Na ions (Brown et al., 1968, 1969, 1970, 1971). Upon termination of illumination the membrane undergoes a long-lasting hyperpolarization; i.e., the membrane potential becomes more negative than that prior to illumination and remains more negative for more than a minute (Koike, Brown, and Hagiwara, 1970). This phenomenon will subsequently be called the postillumination hyperpolarization (PIH). Similar PIH's have been noted to occur in other invertebrate photoreceptors such as in those of *Limulus* (Benolken, 1961; Kikuchi, Naito, and Tanaka, 1962) and dragonfly ommatidial cells (Naka, 1961);

however, the membrane mechanism of PIH has not been fully analyzed. The present paper deals with this mechanism.

The experimental results obtained suggest that the PIH in the barnacle photoreceptor is produced by a mechanism previously referred to as an electrogenic Na pump (Kerkut and Thomas, 1965; Adrian and Slayman, 1966; Nakajima and Takahashi, 1966; Rang and Ritchie, 1968).

MATERIALS AND METHODS

Specimens of *Balanus eburneus* from Woods Hole, Massachusetts, were used. The preparation and recording procedure have been described previously (Brown et al., 1970). One of the lateral ocelli was isolated and a photoreceptor cell was impaled with two 3 M KCl-filled microelectrodes. The membrane potential was recorded as the potential difference between one internal electrode and an external KCl electrode placed in a continuously flowing saline bath. The second internal electrode was used to polarize the membrane with constant current pulses or to maintain the membrane potential at a desired level (voltage-clamp). Maximum intensity of the light pulses used to stimulate the preparation was about 10^6 lux. Normal barnacle saline consisted of (mM) 462 NaCl, 8 KCl, 20 Ca, and 12 Mg. The solution was buffered with 0.01 M Trisma base neutralized with HCl to pH 7.7. Modification of the normal barnacle saline will be described in the appropriate section in the Results. Most of the experiments were performed at room temperature (24°C).

RESULTS

1. *Postillumination Hyperpolarization*

The membrane potential of a photoreceptor cell attained a steady level after several minutes of dark adaptation. In the present work, this potential will be referred to as the resting potential; the average value obtained from 35 cells was -36.3 ± 6.6 mv (SD). The photoreceptor cell responds to illumination with a depolarizing receptor potential (Brown et al., 1970). This is shown by the records in column A of Fig. 1. A reduction in the intensity (1–4) of a light pulse of constant duration is associated with a reduction in the depolarizing receptor potential. The records in column B (1–4) show the same responses as those illustrated in A but with a higher recording sensitivity and a slower sweep speed. The peak of the depolarizing response produced during illumination is not seen in these records, but following illumination a postillumination hyperpolarization was obtained. The membrane potential became more negative than the initial resting potential and returned slowly to the original value. The peak amplitude of the hyperpolarization, measured from the initial resting potential level, increased with increasing light intensity. This was generally true even though the amplitude of the depolarizing response was saturated at high light intensities. A similar increase in the peak amplitude of the PIH was found when the duration of the light pulse

was increased and the light intensity remained constant. When light pulses were applied repetitively, the PIH summated as shown in record B5 of Fig. 1; i.e. light pulses applied so that the time between them was shorter than the recovery period of the PIH, produced a hyperpolarization greater than that produced by a single pulse.

Although the peak amplitude of PIH varied with the intensity and duration of the light pulse, the time course of the PIH was constant and independent

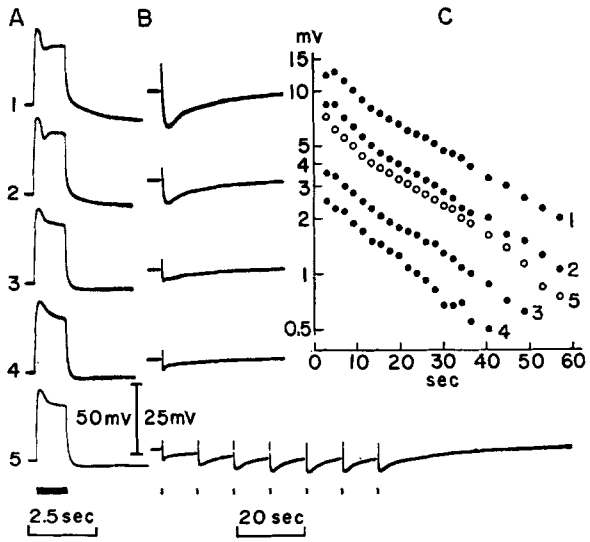

FIGURE 1. Membrane potential changes during and following illumination. A, fast sweep speed to show time course of depolarizing receptor potential. B, slow sweep speed and higher gain to show time course of postillumination hyperpolarization (PIH). The peak membrane potential change during illumination is off-scale at this gain. Light intensity for both series (1 through 4): 10.8 × 10^4 lux, 6.8 × 10^4 lux, 2.5 × 10^4 lux, and 1.9 × 10^4 lux at the surface of the receptor. B5, summation of PIH during repetitive light pulses. Light intensity, 2.5 × 10^4 lux. A5, faster sweep speed record of the first response in the series shown in B5. The solid bars represent the duration of the light pulses. C, amplitude of the PIH measured from the initial resting potential level plotted logarithmically against time following the termination of illumination. Nos. 1 through 4 represent plots from records B1 through B4. The last PIH in the series of B5 is designated 5.

of these parameters. In Fig. 1 C the amplitude of PIH, measured from the initial resting potential level, is shown on a logarithmic scale against the time after termination of illumination. Five relations were obtained from the PIH's illustrated in Fig. 1 (B1–5). For the case of record 5, the PIH was plotted after the last light pulse. The results show that the PIH decays from its peak approximately exponentially and the time constant of the decay is independent of light intensity even following a series of flashes of the same intensity. Similar relations were obtained when the duration of light stimula-

tion was varied at constant intensity. The average time constant calculated for 14 cells at 24°C was 39 ± 13 sec (SD). In some cells (5 out of 14 cells) the PIH decayed in the first 20 sec with a rate substantially higher than the rate found in the following period. This faster rate had an average value of 23 ± 3 sec (SD).

2. *Membrane Conductance during Postillumination Hyperpolarization*

Fig. 2 A shows a depolarizing receptor potential produced by illumination (horizontal bar) followed by a PIH after the illumination was terminated.

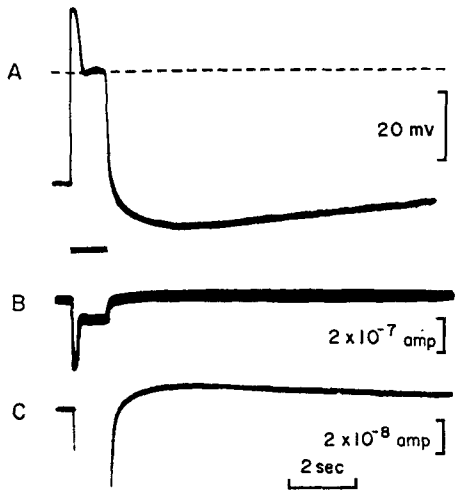

FIGURE 2. Voltage clamp during PIH. A, time course of membrane potential changes during and following a bright step of illumination (horizontal bar) in the absence of voltage clamp. Dotted line represents the reference potential. B, membrane current during and after illumination when the membrane potential was clamped at the resting potential level. A downward deflection of the current trace corresponds to an inward direction of the membrane current. C, same as B except higher gain to better illustrate the outward current following illumination. High frequency noise was reduced by a low pass filter in record C.

The record in Fig. 2 B was obtained from the same cell when a light pulse of the same intensity and duration was applied to the photoreceptor while the membrane potential was clamped to the resting level. An inward membrane current corresponded in time with the depolarizing receptor potential when the membrane was not voltage-clamped. Record C, obtained with higher amplification, shows a postillumination outward current that corresponds to the PIH. In order to examine the membrane conductance during the post-illumination current, a train of short voltage pulses of small amplitude was added to the commanding voltage of the voltage-clamp circuit and the membrane resistance at a given time during and following illumination was

obtained from the ratio of the amplitude of the applied voltage pulses and the corresponding change in the membrane current just after the capacitative surge. This ratio as a function of time is shown in Fig. 3. The membrane resistance decreased significantly during illumination (see Brown et al., 1969, 1970); at the termination of the light pulse the membrane resistance recovered rapidly to the original level prior to illumination. A slight decrease was seen up to 2 sec after termination of light, but no detectable decrease was found by the present method in the following period. Since the PIH or the corresponding outward current under voltage-clamp was maximal 2–3 sec after the termination of light and lasted for several minutes, the PIH does not seem to be associated with any significant increase of membrane conductance; i.e., the potential change does not seem to be due to a conductance increase to ions whose equilibrium potential is slightly more negative than

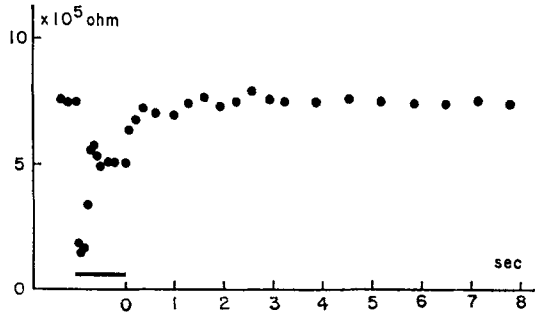

FIGURE 3. Membrane resistance during and following illumination. The ratio of a brief step change in the membrane potential and membrane current is plotted against time. A short time after the beginning of the step changes, a light pulse was applied during the period indicated by the horizontal bar. Note that 2 sec after illumination the ratio returns to the same level as that prior to illumination.

the resting potential. From the analyses of current-voltage relations obtained during the PIH, a small "apparent" increase of the membrane resistance is found during PIH. This will be discussed later.

3. *Effect of Ouabain on PIH*

Fig. 4 shows the effect of ouabain upon PIH. Record A was obtained with a bright light during perfusion with normal saline. The depolarizing receptor potential was followed by a large PIH; at the peak of the PIH the membrane potential reached about −100 mv. Addition of ouabain to the perfusate at a concentration of 10^{-5}M for 20 min totally abolished the PIH (record B); at this stage, there was no significant change in the depolarizing receptor potential. Ouabain did produce a slight decrease of the resting membrane potential (about 6 mv). Removal of ouabain from the perfusate usually resulted in but a slight recovery of PIH. If the preparation was bathed with

10^{-5}M ouabain for a prolonged period or if the ouabain concentration was raised to 10^{-4}M, the PIH and the depolarizing receptor potential were both abolished (Smith et al., 1968). However, under these conditions, the resting membrane potential was reduced by only 6–7 mv even though there was total abolition of the two types of responses.

4. *Effect of Temperature on PIH*

A reduction in the temperature of the bathing medium reduced the amplitude of PIH. Fig. 5 shows the relationship between the peak amplitude of PIH and the temperature when light pulses of constant intensity and duration were applied to the same cell at various temperatures. The result shows

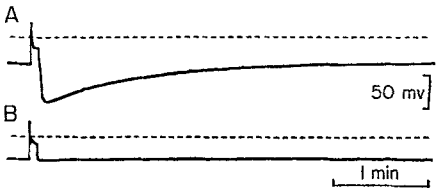

FIGURE 4. Effect of ouabain on PIH. A, a large PIH following depolarization of the membrane by a bright step of light. B, response to light of the same intensity and duration following 20 min in a bathing solution containing 10^{-5} M ouabain.

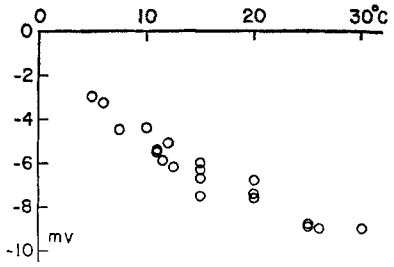

FIGURE 5. Relation of PIH and temperature. The amplitude of PIH, following a light pulse of fixed intensity and duration, plotted against temperature in degrees C.

that Q_{10} is about 2.2 for the range of temperature between 5 and 15°C and about 1.3 for that between 15 and 25°C. In these experiments, the amplitude of the depolarizing receptor potential was not altered significantly because light pulses of high intensity were used; i.e., the amplitude of the depolarizing response was almost at the saturation level.

5. *Effect of External and Internal Na Ions on PIH*

In the experiment illustrated by Fig. 6, the total NaCl concentration (462 mM) in the normal external saline was replaced with an equivalent amount of LiCl. Trace A1 shows that the response to a light pulse in normal saline

consisted of a depolarizing receptor potential followed by a marked PIH after illumination. There was a positive shift of the resting potential by about 7 mv upon replacement of Na with Li (B1). The depolarizing response to a light pulse of the same intensity and duration became much smaller especially during the steady phase of the response and the PIH was totally abolished. These changes were, as a rule, reversible as shown by record C1. The reduction of the amplitude of PIH in C1 was probably due to deteriora-

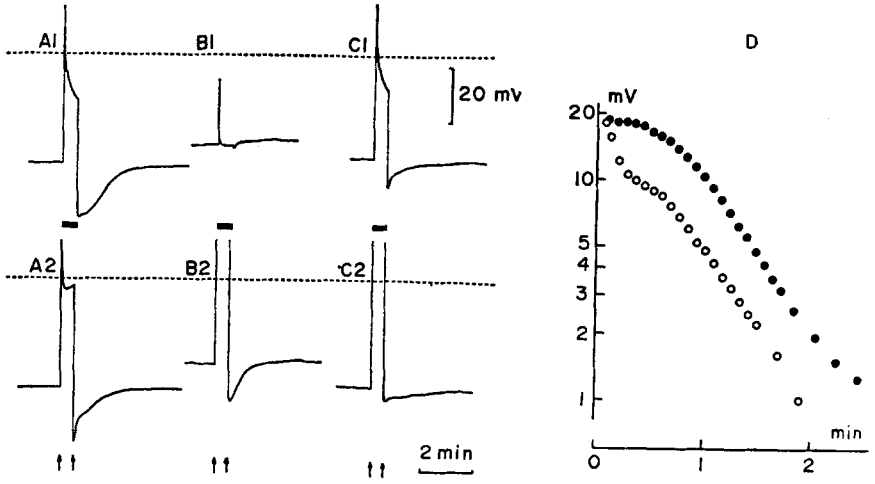

FIGURE 6. Effect of reduced external Na and increased internal Na on PIH. PIH produced following a bright step of light (bar in A1) and following injection of Na through a microelectrode inside the cell (between arrows in A2). During the time of Na injection the membrane was depolarized by the injecting pulse. B1 and B2, same as A1 and A2, respectively except that Na was replaced with Li in the external solution. C1 and C2, control responses in normal saline following Li saline. D, amplitudes of PIH (solid circles) and post-Na-injection potential changes (open circles) plotted logarithmically against time following termination of light and Na injection. The relations are from records A1 and A2.

tion of the membrane caused by prolonged insertion of the electrode. Record A2 in Fig. 6 was obtained in the Na medium shortly after record A1 was taken. Na ions were injected into the cell electrophoretically through Na-filled micropipettes during the period indicated by the arrows. Termination of the injection was followed by a hyperpolarization which rapidly attained a peak and then decayed slowly to the previous resting level. B2 shows the membrane potential changes produced by Na injection in Na-free, Li medium. A similar hyperpolarization was produced. In Fig. 6D, amplitudes of the hyperpolarizations produced by light (solid circles) and Na injection were plotted on a logarithmic scale against the time after termination of these procedures. The result shows that both types of hyperpolarization decay with almost

identical time constants, suggesting that the hyperpolarizations that followed Na injection and illumination may have a common mechanism. The influx of Na ions during the depolarizing receptor potential (Brown et al., 1970) may initiate the PIH. This would explain the abolition of PIH in Li media shown in Fig. 6 (B1) since there should be no Na influx in Na-free media. If Na influx, and hence an increase of the inernal Na concentration, is the cause of the PIH, a similar hyperpolarization should be obtained by injection of Na ions even in a Na-free medium. Such was the case as shown in B2; i.e., an electrophoretic injection of Na ions produced a prolonged hyperpolarization just as it did in a normal Na medium. The smaller hyperpolarization following Na injection after the preparation had been returned to the normal Na saline (C2) was probably due to some sort of deterioration; this corresponds to the smaller PIH in record C1.

6. *Effect of External K on PIH*

Fig. 7 A shows summation of PIH's when light pulses of about 1 sec duration were repeated at 10 sec intervals. Fig. 7 A′ illustrates a record obtained with a faster sweep speed during and following a single light pulse. When the external K ions (8 mM) were removed by replacing the KCl in the normal saline with an osmotically equivalent amount of Tris-chloride (Fig. 7 B), the resting potential became slightly less negative (about 5 mv). This was a consistent finding. Although the depolarizing receptor potential remained vir-

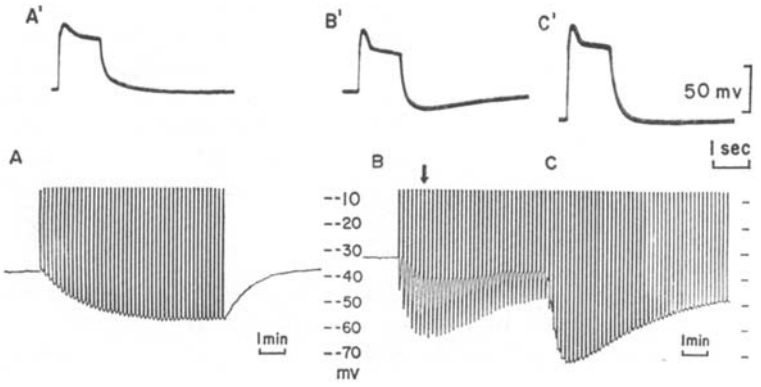

FIGURE 7 A. Summation of PIH obtained in normal saline. Responses produced by repetitive application of light pulses. Peaks of the depolarizing responses are not seen in the record. A′ shows potential changes to the first light pulse in the series. B, same as A when all the KCl in the normal saline had been replaced by Tris-chloride. B′ from series B at the time indicated by the arrow. Note the faster recovery of the membrane potential from the peak of PIH. C, the recovery of PIH when the cell was returned to normal saline. C′, one of the responses obtained after the cell was back to normal saline.

tually the same, marked changes occurred in PIH. The time constant of decay was altered from about 60 sec (A) to about 4 sec (B′) in the K-free medium. As a result of this, summation of PIH's during successive light pulses became much less pronounced. The peak amplitude of PIH increased for the several initial pulses, but following this, gradually decreased until a small steady value was attained. At the time indicated by the letter C, the preparation was returned to the normal saline. The time course of the PIH recovered to the original value and its peak amplitude transiently increased to a greater value than that observed in the previous normal saline; gradually the amplitude decreased to a steady level similar to that reached during successive light pulses in normal saline. The fact that this level in C is less negative than that seen in A is probably due to some deterioration of the cell membrane. The marked transient increase in the peak amplitude of PIH following the introduction of normal K solution resembles the phenomenon of "K activation" of the electrogenic Na pump that has been described previously in other cells (Rang and Ritchie, 1968; Carpenter and Alving, 1968; Den Hertog and Ritchie, 1969).

7. *Charge Transfer during and following Illumination*

Records A1 through C1 in Fig. 8 show membrane currents when the membrane potential was clamped at the resting level and light pulses of three different intensities were applied. Light-induced inward currents occurred during the light pulse. It was shown previously (Brown et al., 1970) that the light-induced inward current is mainly carried by Na ions. Records A2 through C2 show recordings of the same responses but at higher sensitivity and a slower sweep speed in order to show the postillumination outward current. If it is assumed that this current is driven by an electrogenic Na pump activated by the internal increase in Na concentration during illumination, the quantity of charge transferred from the inside to the outside of the membrane should be related to the amount of inward charge transfer during illumination. The inward and outward charge transferred during and after illumination was calculated from recordings such as those shown in Fig. 8 A-C when the duration and intensity of light were varied. The relation between the two quantities was almost linear as shown by the plot in Fig. 8. The slope of the relation indicates that the ratio between the inward and the outward charge transfer is close to unity. This immediately suggests that all the Na ions extruded by the pump are electrogenic or that the electrogenicity of the pump is complete. However, the actual Na entry during illumination could be greater than that calculated from the net inward charge transfer if there is a significant countercurrent carried by ions other than Na ions. Furthermore, as described previously (Brown et al., 1971), the three photoreceptor cells in the lateral eyes are electrotonically coupled. Therefore, the

current recorded during voltage-clamp experiments represents not only that flowing through the membrane of the voltage-clamped cell (primary current), but also that flowing through other cell membranes (secondary current). The proportion of the secondary current to the primary current flowing during illumination is small because of the high membrane conductance of the

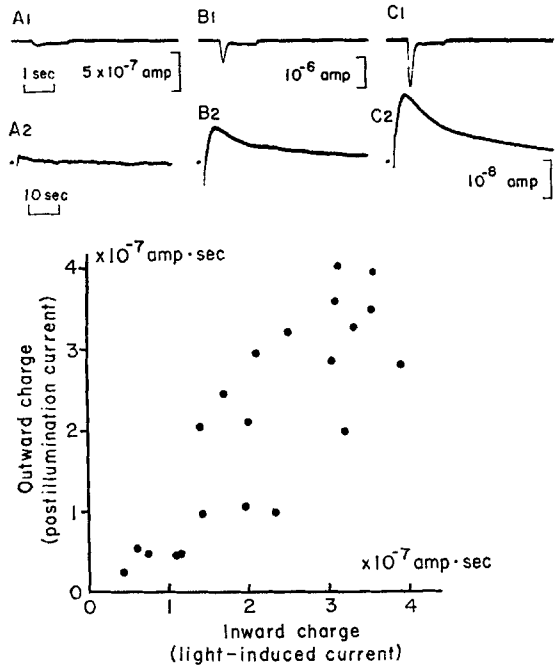

FIGURE 8. Relation of membrane current during and following illumination. A1 through C1. Low gain records to show inward membrane current during light pulses of increasing intensity when the membrane potential was clamped at the resting level. A2 through C2. Higher gain recordings at a slower sweep speed to show outward membrane current following the same three light pulses. Bottom, relation between outward charge transfer following illumination and inward charge transfer during illumination (time integral of membrane current). Data were obtained by varying both the duration and intensity of the light pulse.

photoreceptor during illumination. However, following illumination and during the postillumination outward current, the secondary current may become proportionately greater since the membrane conductance is low during this phase of the response. The above considerations suggest that the ratio between the quantity of Na ions extruded by the electrogenic Na pump and the quantity entering could be significantly smaller than unity but it is nevertheless certain that there is an almost linear relation between them.

8. *Current-Voltage Relations of the Membrane during PIH*

In order to obtain current-voltage relations of the membrane during PIH, inward and outward current pulses of various intensities were applied at a time corresponding to the peak of PIH (Fig. 9). The duration of the current pulse was about 1 sec and the intensity and duration of the light pulse remained fixed; measurements for the relation were made at a time prior to termination of the pulse. The result shown in Fig. 10 A (open circles) is a plot of the relation between the absolute membrane potential and the inten-

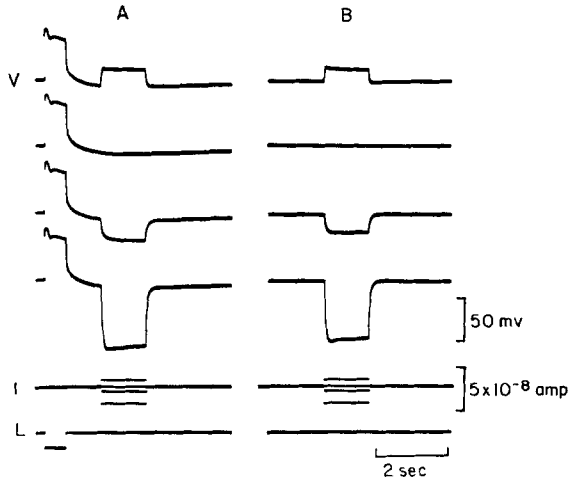

FIGURE 9. PIH during current clamp conditions. A, membrane potential changes (V) associated with current of various strengths (I) applied during the PIH that followed illumination (L). B, membrane potential changes associated with various strengths of current in the absence of illumination.

sity of applied current. The relation illustrated by solid circles was obtained when the membrane was in the resting state (Fig. 9 B). Both relations are nonlinear.

Since the current-voltage relations were obtained practically at the steady state, the membrane currents are considered functions of the membrane potential. When the applied currents during PIH and at rest are denoted by $I_{PIH}(V)$ and $I_R(V)$, respectively, $\Delta I(V) = I_{PIH}(V) - I_R(V)$. ΔI for a given V should represent the amount of postillumination outward current obtained under the voltage-clamp of the membrane at V. ΔI's were calculated from Fig. 10 A for each V and are shown plotted on the abscissa against V on the ordinate in Fig. 10 B. The relation shows that the amplitude of the postillu-

mination outward current is almost constant when the membrane potential is more negative than −80 mv, but at more positive membrane potentials it decreases approximately linearly. Extrapolation of this relation to $\Delta I = 0$ yields a membrane potential between 0 and +10 mv. This result suggests the possibility that PIH is produced by a conductance decrease in series with a fixed emf. In other words, the membrane is equivalent to two parallel conductances each in series with an emf. The one emf has a large negative value; for example −100 mv, and the other a value between 0 and +10 mv. Both conductances are almost comparable in the resting state or in the dark so that the resting potential is at a value between them, e.g. −40 mv. At the termination of light the conductance in series with the positive emf decreases so that a hyperpolarization occurs. This mechanism is similar to the one proposed for the receptor potential in the vertebrate retina (Toyoda, Nosaki,

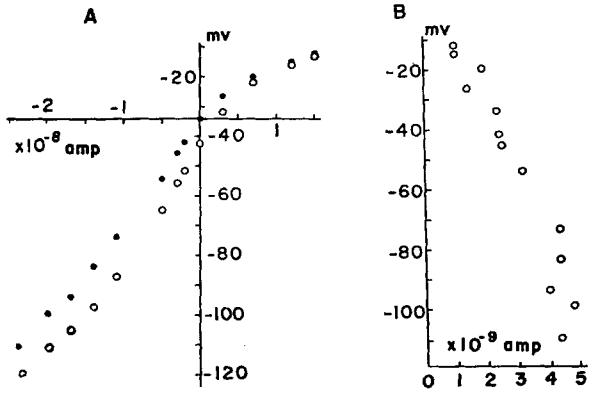

FIGURE 10. Voltage-current relations of the membrane during PIH. A, open circles represent the relation during PIH (Fig. 9 A) and solid circles show the relation obtained in the resting state (Fig. 9 B). B, plot of $\Delta I(V)$ from Fig. 10 A.

and Tomita, 1969; Baylor and Fuortes, 1970). Since the membrane potential at the peak of PIH can reach −100 mv (Fig. 4), one of the emf's in this model should be at least more negative than −100 mv and this could be represented by the potential determined by the K concentration gradient across the membrane. If this is the case, the above model requires a relatively large conductance to some species of ions with an equilibrium potential around 0 to +10 mv. The fact that changes in Na, Ca, Mg, as well as in Cl concentration in the external saline resulted in insignificant changes of the resting potential suggests that the presence of such a conductance is unlikely. If it is accepted that PIH is produced by an electrogenic Na pump, the present data indicate that either the electrogenicity of the pump depends on the membrane potential or that the pump activity is associated with a change of membrane con-

ductance (Geduldig, 1968). At the present stage it is difficult to distinguish between these alternatives.

DISCUSSION

Although there is some evidence (Fig. 10) that the PIH is produced by a conductance decrease similar to the one proposed for vertebrate photoreceptors (Toyoda et al., 1969; Baylor and Fuortes, 1970), the majority of the evidence indicates that the PIH is most probably an expression of an electrogenic Na pump. To summarize: (*a*) There is no increase in membrane conductance of sufficient magnitude during PIH to explain the potential change as the result of an increase in permeability to some ion whose equilibrium potential is slightly more negative than the resting potential. (*b*) PIH is abolished by ouabain at a time when the depolarizing response is still intact. (*c*) The amplitude of the PIH has a relatively large Q_{10}. (*d*) Intracellular injection of Na ions produces a hyperpolarization with a similar time course. (*e*) Removal of K from the external saline reduces the amplitude of PIH and a transient increase of the PIH occurs when the preparation is returned from K-free solution. The shorter time course of PIH in K-free solution may be explained by a transient increase in Na pump activity produced by a temporary increase of K ions just outside the membrane during depolarization of the membrane or by a brief increase of membrane permeability to K ions just after termination of the light (Fig. 3). (*f*) Preceding work (Brown et al., 1970, 1971) showed that the depolarizing response is produced by an increase in permeability of the membrane mainly due to Na ions. Therefore, a significant Na influx is expected during illumination. (*g*) PIH is abolished in Na-free media. This indicates that the electrogenic Na pump is not directly triggered by illumination but by an increase of the internal Na concentration due to the Na influx during the depolarizing response. These results are consistent with those reported for similar long-lasting afterpotentials in other preparations (Kerkut and Thomas, 1965; Nakajima and Takahashi, 1966; Adrian and Slayman, 1966; Rang and Ritchie, 1968; Thomas, 1969).

Although it has been reported that light may alter the electrogenicity of the Na pump in *Limulus* photoreceptors (Smith et al., 1968), there was no evidence for this in barnacle photoreceptors. Light appears to play a role only in increasing membrane permeability to Na ions (Brown et al., 1970) which in turn probably activates the electrogenic pump (Fig. 9).

The PIH may play a functional role in the behavior of the barnacle. Gwilliam (1963) observed that nerve discharges from supraesophogeal ganglion cells, the presumed site of termination of photoreceptor cell axons, were facilitated at the close of a pulse of illumination. This facilitation coincides approximately with the time course of the PIH reported in the present paper.

During the time of illumination this activity appears to be inhibited. The photoreceptor cell axons normally do not conduct action potentials and there is evidence of electrotonic spread of the receptor potential to the second-order neurons (Gwilliam, 1963, 1965). This may imply that the depolarizing receptor potential in some way produces inhibition of the second-order neurons or that the PIH in some manner produces activation of the second-order terminals.

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