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DUAL ROLE FOR POTASSIUM IN *BALANUS* PHOTORECEPTOR: ANTAGONIST OF CALCIUM AND SUPPRESSION OF LIGHT-INDUCED CURRENT

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SUMMARY

1. The mechanism of reduction and final abolition of the depolarizing receptor potential of *Balanus eburneus* photoreceptors in K^+-free saline was examined with electro-physiological techniques including voltage-clamp and ion specific electrodes.

2. An extended exposure to K^+-free saline reduces the transient peak and the steady phases of the depolarizing receptor potential by approximately equal amounts. The process can be reversed in normal saline although the wave form of the response is often more rectangular upon recovery. Restoration of K^+ induces a transient hyperpolarization of the resting membrane for several minutes.

3. The depolarizing receptor potential can also be restored in K^+-free solution by reducing the Ca^{2+} concentration. This saline depolarizes the resting membrane, and the wave form of the depolarizing receptor potential assumes a rectangular configuration.

4. Voltage-clamp experiments revealed that an extended exposure to K^+-free saline produced an extreme reduction of the inward light-induced current (LIC), but no detectable change in the membrane potential at which the current reverses sign. Membrane conductance in darkness showed little change. Reduction of the Ca^{2+} concentration from 20 to 0·2 mM in K^+-free restored the current and produced a negative 8–10 mV shift in the zero current potential. There was also a significant decrease in membrane conductance in darkness.

5. Current–voltage relations of the membrane in K^+-free, low Ca^{2+}, or K^+-free low Ca^{2+} salines were somewhat dependent upon the order the salines were presented.

6. Low Ca^{2+} saline (0·2 mM) by itself produced a -5 mV shift in the

zero-current potential. Removing K^+ in low Ca^{2+} produced an additional shift (-5 mV) in the zero-current potential.

7. Intracellular Na^+ and K^+ activities (a^i_{Na}, a^i_K) were monitored with Na^+-sensitive glass and K^+-sensitive liquid ion-exchanger electrodes in the above salines.

8. Removal of K^+ from the saline was associated with a large increase in a^i_{Na} and a decrease in a^i_K over a period of 30 min. Reduction of Ca^{2+} had little effect on a^i_{Na} or a^i_K over the same time span, but reduction of Ca^{2+} in K^+-free facilitated the changes observed in K^+-free solutions.

9. Increasing the K^+ in low Ca^{2+} saline can suppress the inward light induced current but is without effect when a normal amount of Ca^{2+} is present. The relation between LIC and K^+ concentration appears to be linear and the relations are parallel in different Ca^{2+} concentrations.

10. It is concluded that some reduction of the depolarizing receptor potential in K^+-free saline is attributable to a reduced driving force for the light-induced current due to an increase in a^i_{Na}. However, the major effect of K^+-free saline on this current appears to originate at a membrane site associated with Ca^{2+}. In the absence of K^+, Ca^{2+} can almost totally suppress the light-induced current, while a normal amount of K^+ antagonizes Ca^{2+} suppression at this site. However, at low Ca^{2+} concentrations and high K^+ concentration, K^+ ions can also suppress the LIC. Thus, both K^+ and Ca^{2+} exert control on the Na^+ current in this receptor and thereby can significantly modify the depolarizing receptor potential. This mechanism is likely involved in adaptation of the photoreceptor during light stimulation.

INTRODUCTION

The receptor potentials evoked by step stimuli of different types have similar wave forms in slowly adapting receptors such as the photoreceptor in the barnacle (Brown, Meech, Koike & Hagiwara, 1969; Brown, Hagiwara, Koike & Meech, 1970), chemoreceptors in insects (Boeckh, Priesner, Schneider & Jacobson, 1963), mechanoreceptors like the muscle spindle (Husmark & Ottoson, 1971*a*; Ottoson & Shepherd, 1971), or the decapsulated Pacinian corpuscle (Ozeiki & Sato, 1965; Loewenstein & Mendelson, 1965). Generally, the response consists of a transient peak phase followed by a steady phase that is sustained for the duration of the stimulus period. It has been shown in most end organs so far studied that removal of Na^+ has the same general effect: a marked reduction of the response without abolishing it entirely. These similarities suggest that the ionic events underlying the production of the receptor potential are closely similar in these end organs. In studies on the receptor potential of the isolated frog spindle (Husmark & Ottoson, 1971*a*, *b*) it was found that

prolonged exposure of the spindle to K^+-free solution produced a progressive decline of the response. The wave form of the receptor potential underwent interesting changes at the same time. The most remarkable were abolition of the transient overshoot and a slowing of receptor repolarization to the resting level after release of stretch.

In a previous study it was shown that K^+-free solutions applied for a relatively brief time (up to 15 min) produced no appreciable changes in the amplitude or wave form of the depclarizing receptor potential of the barnacle photoreceptor (H. M. Brown *et al.* 1970). However, the membrane potential changes following illumination; the post-illumination-hyperpolarization was significantly reduced in K^+-free saline (Koike, Brown & Hagiwara, 1971). In the present study, attention has been focused on the changes occurring during longer exposures to K^+-free solutions. Under these conditions, the receptor potential can be abolished (Brown & Ottoson, 1973). Results from the present study indicate that K^+ has a dual role in production of the receptor current: (1) it antagonizes the suppression of light induced current exerted by Ca^{2+} and (2) acts as a suppressor in the absence of Ca^{2+} ions.

METHODS

Recording methods and light stimulation have been described previously (Brown *et al.* 1970). Barnacle saline consisted of (mM): NaCl, 462; KCl, 8; $CaCl_2$, 20; $MgCl_2$, 12; Tris, Tris-HCl 10 (pH 7·65).

KCl was replaced in the normal barnacle Ringer by an equivalent amount of NaCl. Other modifications of the barnacle saline are described in the appropriate experimental section. Solution changes occurred rapidly in the chamber (0·2 ml.) with the flow rate used (2·5 ml./min).

Liquid ion-exchanger micro-electrodes were constructed to measure K^+ activities (Corning exchanger 477317) as described by Orme (1969) and Walker (1971). The selectivity of these electrodes for several competing ions of possible significance has been determined (Orme, 1969; Brown, Walker & Sutton, 1970; Walker, 1971; J. H. Saunders & H. M. Brown, unpublished). Na-sensitive electrodes were constructed from Corning NAS 11-18 glass (Eisenman, 1969) according to the method of Thomas (1969) and were generally of the recessed tip variety; tip diameters (*ca.* 1μm) were larger than the exchanger electrodes (*ca.* 0·5 μm). The selectivity coefficient ($k_{Na,K}$) of these electrodes for K^+ to Na^+ was routinely determined from reciprocal dilution of K^+ and Na^+ (as chlorides) at constant ionic strength (0·1 M) to correct for K^+ errors in intracellular Na-activity. The potential difference between the ion electrode and the KCl-filled micro-electrode ($V' - E_m$), was used to calculate the internal ion-activity, a_x^i ($x = Na^+$ or K^+) from the relation:

$$a_x^i = a_x^o . \exp \frac{(V' - E_m) - V}{M} - k_{xy} a_y^i, \qquad (1)$$

where V is the potential of the ion-electrode in the external saline with primary ion-activity a_x^o; M is dV/da_x obtained from calibration of the electrode in pure solutions of the primary ion-species; k_{xy} is the selectivity of the electrode for the competing y ion with respect to the primary ion x, and a_y^i is the internal activity

of the competing ion. The a^{i}_{Na} measurements were routinely corrected by an appropriate amount depending upon the $k_{Na,K}$ obtained experimentally for a given Na-electrode. These values ranged from 0·01 to 0·005 and $k_{K,Na}$ for K electrodes ranged from 0·02 to 0·01.

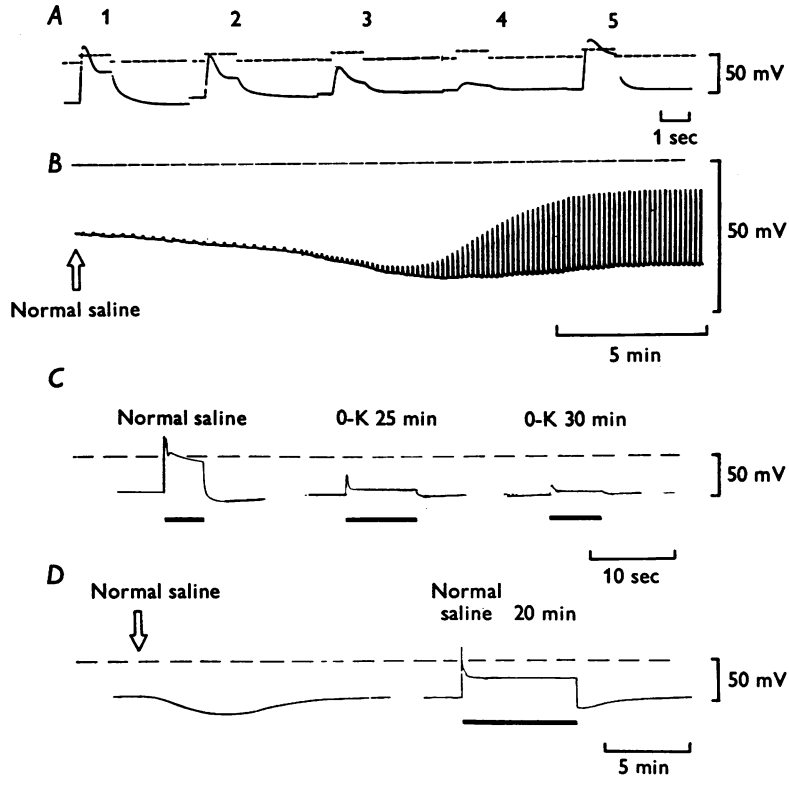

Fig. 1. Membrane potential changes of barnacle photoreceptor during and following extended exposure to K^+-free saline. *A*, membrane potential changes produced by 1 sec pulses of light (1/10 sec) of fixed intensity and duration at various times after removal of K^+: *A*1, control in normal barnacle saline; *A*2, 30 min; *A*3, 45 min; *A*4, 60 min; *A*5, 20 min in normal saline. Interrupted line in the record represents bath potential; an upward excursion of this line represents onset of illumination. *B*, different cell, record on a slow time base to show changes in the resting potential and depolarizing receptor potential when K^+-containing saline was reintroduced to the bath; 1 sec light pulses were being applied. *C*, membrane potential changes to long steps of intermittent illumination at different times (indicated) after 0-K saline. *D*, same cell; resting membrane potential change in darkness following introduction of K^+-containing barnacle saline. After 20 min in this saline the response to light had fully recovered (right).

RESULTS

Effect of K⁺-free saline on resting and receptor potential

Changes of the depolarizing receptor potential (DRP) after different periods of time in K^+-free solution are illustrated in Fig. 1. Usually after 20–30 min there was a progressive decline in amplitude of the depolarizing potential change to light (*A* and *C*); with a sufficiently long exposure, the response was completely abolished. In general, the transient phase and the steady phase of the response were reduced by about the same amount (Fig. 2).

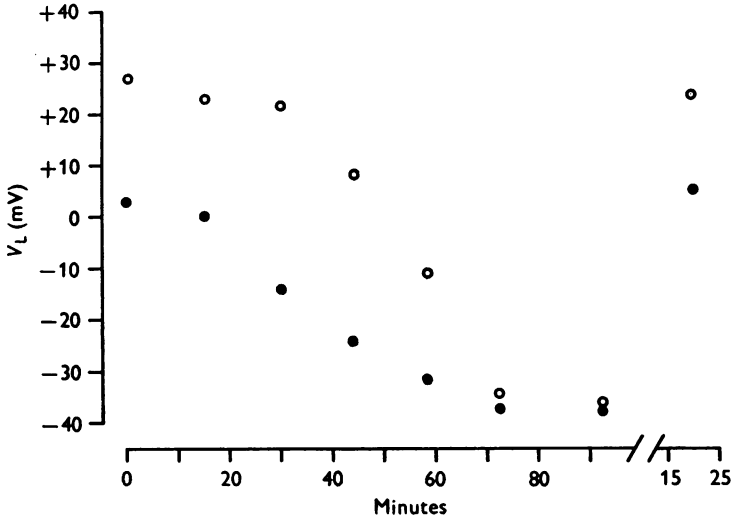

Fig. 2. Membrane potential of the light response (V_L) at the peak (○) and steady (●) phase at different times (min) following K^+ removal. Intense, white, 1 sec flashes were presented to monitor the depolarizing receptor potential. After 1½ hr in 0-K saline the receptor potential was totally abolished ($V_{peak} = V_{steady} = E_r = -40$ mV). Within 20 min in normal saline (break in abscissa), there was almost total recovery of the response to light.

During repetitive light stimulation (1/10 sec) in the absence of K^+ (Fig. 1*A*) there was an apparent decline in the resting potential. At this stimulation frequency the post-illumination hyperpolarization (Koike *et al.* 1971) can contribute to the resting potential, and since 0-K^+ abolishes the post-illumination hyperpolarization the resting potential appears to be reduced. In experiments with intermittent light stimulation, the resting potential declined by only 3 or 4 mV, but the same reduction in the depolarizing receptor potential was observed.

Provided that K^+ was returned to the bath before the response was

completely abolished, the response to light usually recovered within 15–20 min as indicated in Fig. 1*A* and *D*, bottom right. The amplitude of the steady phase of the response usually recovered to within a few percent of the original value and in some cases actually exceeded the control level (record 5). On occasion the peak phase did not fully attain the control value and the response often assumed a rectangular wave form upon return to normal saline; this remained unchanged even after prolonged exposure to normal saline.

The resting potential changed significantly when normal saline was introduced after the prolonged exposure to K^+-free saline; this change occurred in the presence or absence of illumination as shown in records *B* and *D* of Fig. 1. There was an initial hyperpolarization of the membrane that lasted several minutes, then the membrane potential slowly returned to about the same level as that before removal of K^+.

Low-Ca^{2+} reverses the K^+-free effect

Following reduction of the DRP in K^+-free solution, the receptor potential could be restored if Ca^{2+} was reduced in the external saline as shown in Fig. 3. Repetitive light flashes were applied and K^+ was removed from the bath (arrow). This resulted in reduction of the receptor potential and an apparent decrease in the resting potential. Panel *B* shows a record after 20 min in K^+-free saline. The depolarizing receptor potential was reduced to about 20 % of its initial value and there was an apparent decrease in the resting potential of approximately 10 mV. At the arrow the Ca^{2+} concentration was reduced from 20 to 0·2 mM. This resulted in recovery of the depolarizing receptor potential within 5 min after the Ca^{2+} was reduced. Reducing the Ca^{2+} concentration in the absence of K^+ always produced a further reduction in the resting potential, i.e. the prevailing membrane potential between light flashes, and led to a pronounced but brief post-illumination hyperpolarization; this is best seen in panel *C*. The membrane potential during the post-illumination hyperpolarization was about the same as the membrane potential between light flashes before application of the low Ca^{2+} solution. Even though the amplitude of the depolarizing receptor potential was restored in the low Ca^{2+} saline the configuration of the response changed significantly. Prior to the low Ca^{2+} saline, there was typically a peak transient and a steady phase of reduced amplitude; in reduced Ca^{2+} the light response became more rectangular. Restoration of K^+ to the medium resulted in an immediate hyperpolarization of the resting membrane by more than 15 mV (panel *C*). Concomitant with this change was a reduction in amplitude of the post-illumination hyperpolarization. The initial hyperpolarization is the K-activated component shown to occur in darkness

or during repetitive illumination (Fig. 1). Maintenance of this membrane potential level is mostly attributable to summation of the tails of post-illumination hyperpolarization due to their increased time course. The peak amplitude of the receptor potential was only slightly reduced upon return to K^+ in the low Ca^{2+} saline and the wave form assumed an even more rectangular configuration.

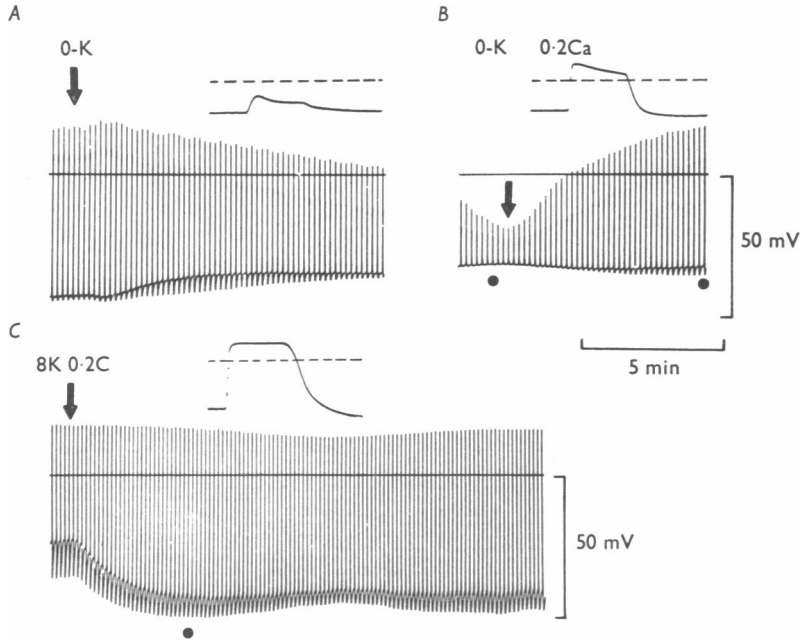

Fig. 3. Reversal of the 0-K effect on the depolarizing receptor (DRP) upon reduction of the external Ca^{2+} concentration. 1 sec pulses of light presented every 10 sec. *A*, removal of the K^+ depolarizes membrane and leads to progressive diminution of the light response. A brief post-illumination hyperpolarization (PIH) appears transiently following removal of K^+. *B*, 10 min after record *A*. The resting potential is at a new steady state. The receptor potential still consists of a transient peak and steady phase although both are significantly reduced in amplitude. The inset in *A* shows the depolarizing receptor potential indicated by the left dot in panel *B*. Reducing Ca^{2+} from 20 to 0·2 mM (arrow in *B*) restores the receptor potential within 5 min; a transient undershoot (brief post-illumination hyperpolarization) again develops. Inset shows the depolarizing receptor potential obtained at the right dot. *C*, 10 min in K^+-free, 0·2 ca^{2+} saline. Membrane has depolarized further and there is a large transient undershoot following the light flashes. Restoration of K^+ is indicated by the arrow. The membrane hyperpolarizes and post-illumination hyperpolarization becomes longer. The membrane potential during illumination remains about the same as in the 0-K, low Ca^{2+} solution and the configuration of the response becomes more rectangular.

Fig. 4 demonstrates that the potential changes observed in low Ca^{2+} saline (Fig. 3) were not simply due to an extended exposure to the K^+-free saline. The same membrane phenomena were evident when K^+ and Ca^{2+} were reduced simultaneously as when there was an intermediate exposure to K^+-free saline followed by a reduction in Ca^{2+}. Simultaneous reduction of Ca^{2+} and K^+ produced an immediate reduction in the apparent level of the resting potential and augmentation of both the peak and steady

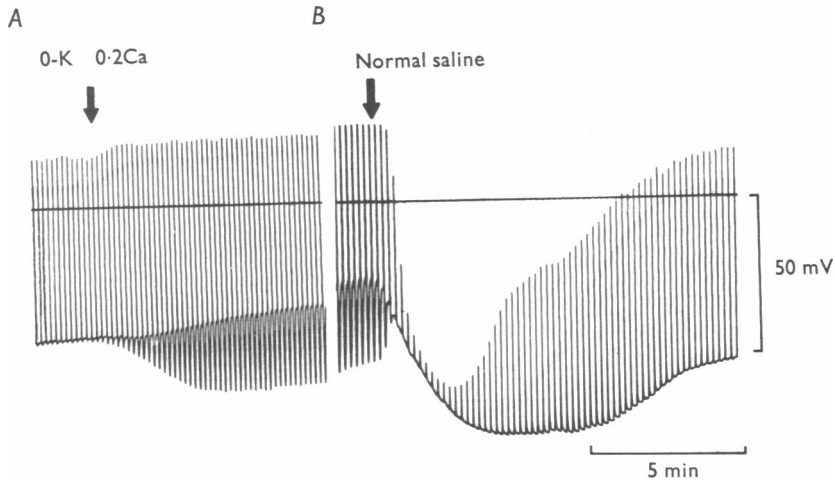

Fig. 4. Effects of simultaneous reduction of K^+ (K-free) and Ca^{2+} (0·2 mM). *A*, membrane depolarizes and a large transient post-illumination hyperpolarization develops. This is sustained during prolonged application of the solution. The depolarizing receptor potential is augmented and receptor potential becomes more rectangular. *B*, upon return to normal saline, the membrane hyperpolarizes by more than 30 mV, the depolarizing receptor potential is almost totally abolished, but recovers during the transient hyperpolarization of the membrane. The depolarizing receptor potential again assumes the usual wave form consisting of a transient peak and steady phase (see Fig. 5).

phase of the depolarizing receptor potential. These features were still evident after an extended exposure to this saline; the break in the record between panel *A* and *B* represents 10 min. Upon return to normal saline (panel *B*) the membrane immediately hyperpolarized and the depolarizing receptor potential was almost abolished for more than a minute. The receptor potential then began to recover and this recovery occurred before the time that the resting membrane had hyperpolarized to the maximum extent. The behaviour of post-illumination hyperpolarization is noteworthy under these conditions. PIH was greatly augmented in 0-K, 0·2 Ca (*A*), and abolished upon return to normal saline (*B*). At the nadir of the slow membrane hyperpolarization there was a small positive

after-potential that gradually became a post-illumination hyperpolarization as the slow hyperpolarization declined. There is an early rapid component of PIH associated with an increased K-conductance (Koike *et al.* 1971). As such, these changes are probably associated with changes in E_k under these experimental conditions as shown in Fig. 10.

An extended exposure to low Ca^{2+} saline alone produced most of the same effects on the DRP, but not on the resting potential as seen in Fig. 5.

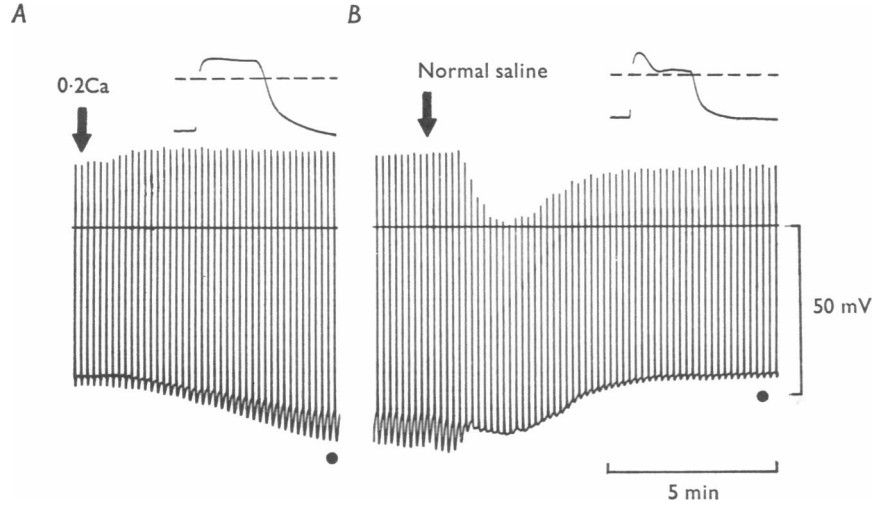

Fig. 5. Effect of low Ca^{2+} saline. *A*, a change from normal barnacle saline to low Ca^{2+} saline (0·2 Ca^{2+}) is accompanied by a membrane hyperpolarization and a slight post-illumination hyperpolarization. The receptor potential increases in absolute voltage and the steady phase attains almost the same voltage as the peak transient phase (inset). *B*, 10 min later. Changes mentioned in *A* are in a steady condition. Returning to the normal saline results in diminution of the resting potential, a transient decrease in the receptor potential and the wave form of the receptor potential regains the usual form (inset). At the maximum of the trough, the peak and steady phase are of equal amplitude and as the receptor potential recovers there is a progressive increase of the steady phase until the control level in normal saline is attained.

The break in the record of this Figure represents 10 min. The low Ca^{2+} saline resulted in an apparent increase in the resting potential level rather than a decrease as shown in Fig. 4. However, the depolarizing receptor potential and the post-illumination hyperpolarization were both augmented as was observed when Ca^{2+} and K^+ were reduced simultaneously and the depolarizing receptor potential assumed the rectangular configuration. Upon return to normal saline (arrow in panel *B*), the DRP was reduced somewhat but the large membrane hyperpolarization that

occurred when K^+ as well as Ca^{2+} was restored did not occur. The light response can be seen to be comprised of the more typical peak and steady phase after replacement of the Ca^{2+} in the saline.

To summarize these initial observations: (*a*) Extended exposure to K^+-free saline abolished the depolarizing receptor potential which can be restored if Ca^{2+} is reduced in the saline. (*b*) Simultaneous reduction of Ca^{2+} and K^+ does not abolish the receptor potential but upon return to normal saline it is almost totally abolished for several minutes. Upon exposure to normal saline following reduced Ca^{2+}, there is a transient reduction in the depolarizing receptor potential although not to the extent seen in the former case. (*c*) During repetitive illumination (1/10 sec), K^+-free solution causes an apparent membrane depolarization whereas the membrane was hyperpolarized in low Ca^{2+} saline. (*d*) Both low Ca^{2+} and K^+-free potentiates the post-illumination hyperpolarization, but K^+-free does so only transiently whereas it is sustained in the low Ca^{2+} saline. (*e*) A K^+-activated membrane hyperpolarization occurs in normal saline after an extended exposure to K^+-free saline. This is likely associated with activation of an electrogenic pump (Koike *et al.* 1971). This K^+-induced hyperpolarization is also observed in low Ca^{2+} saline.

Current–voltage relations in K^+-free and low Ca^{2+} salines

Membrane current–voltage (*I–V*) relations were altered in K^+-free or low Ca^{2+} salines but the alterations were dependent on the sequence in which the salines were presented. The records shown in the top row of Fig. 6 are membrane potential changes to a flash of light in normal, K^+-free, and K^+-free, low Ca^{2+} saline. The voltage-clamp records in K^+-free, were obtained after the DRP was significantly reduced but at a time that there was sufficient light induced current for measurement. Each column shows records of membrane current when the membrane potential was voltage-clamped to the potential indicated to the left of the record. Calibration bars for membrane current apply to each column; note that the gain was increased for the record at +30 mV in 0-K saline. By comparing the records at about −20 mV (second row), it can be seen that there was a significant reduction in the inward light induced current in 0-K saline but light induced current increased in low Ca^{2+} saline in the absence of K^+. The membrane potential at which the light induced current became undetectable was about +30 mV in normal saline and 0-K but the zero-current membrane potential was less positive in 0-K when Ca^{2+} was also reduced (third column). At higher membrane potential levels the light induced current had clearly reversed sign in each of the salines.

I–V relations of the membrane in this sequence of salines are shown in Fig. 7*A*. Membrane current in darkness just before the light flash is

represented by filled symbols and the peak light induced current (I_{total}–I_{dark}) is represented by open symbols. K^+-free saline produced little change of the membrane slope conductance in darkness as seen from the almost parallel relations represented by filled circles and squares. However, when Ca^{2+} was reduced in the absence of K^+, the conductance was reduced to about half of what it was in the other salines. The measured

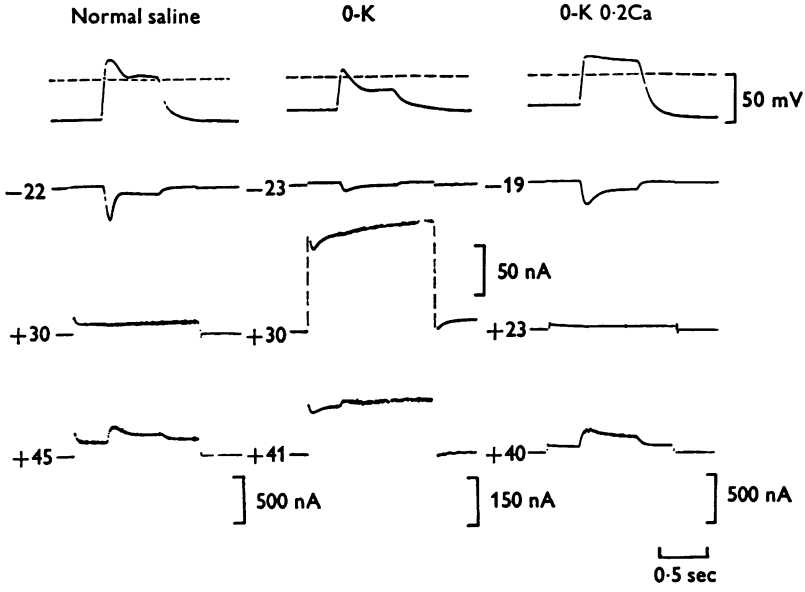

Fig. 6. Voltage-clamp experiment showing membrane current in normal, K^+-free, and K^+-free low Ca^{2+} saline. In the top row are shown membrane potential changes to light in the three salines. Records in the second and third column were not obtained in the steady-state since there was continual reduction in the light-induced membrane current in 0-K, and some decline in the resting potential from the beginning to the end of the series in the 0-K, 0·2 Ca^{2+} saline. In neither case did these changes exceed 10% from the beginning to the end of the series. The holding potential in each case was the membrane potential at the beginning of the voltage clamp series. The command potential was about 1·3 sec in duration; an 0·6 sec light pulse was applied 0·33 sec after the command pulse was initiated. The calibration bars pertain to each column of membrane current records. Note that the gain was increased in the record obtained at +30 mV in the 0-K saline to show that the zero current potential is the same as it was in normal saline.

membrane conductance between 0 and +30 mV was $2{\cdot}33 \times 10^{-6}$ mhos in normal saline and 0-K and $1{\cdot}03 \times 10^{-6}$ mhos in K^+-free, low Ca^{2+} saline. The most dramatic effect of the K^+-free saline was on the light-induced current as seen by comparing the open circles and squares. At −40 mV

the light-induced current was reduced to only about 10 % of the value in the normal saline. Even so, this current reversed sign at about the same membrane potential as it did in normal saline. When Ca^{2+} was reduced one hundredfold in the absence of K^+, the light induced current at E_m −40 mV recovered to about 75 % of the value in the normal saline and the slope conductance at the reversal potential was almost identical to the conductance observed in normal saline. However, the membrane potential at which light-induced current reversed sign was shifted from +30 to +23 mV.

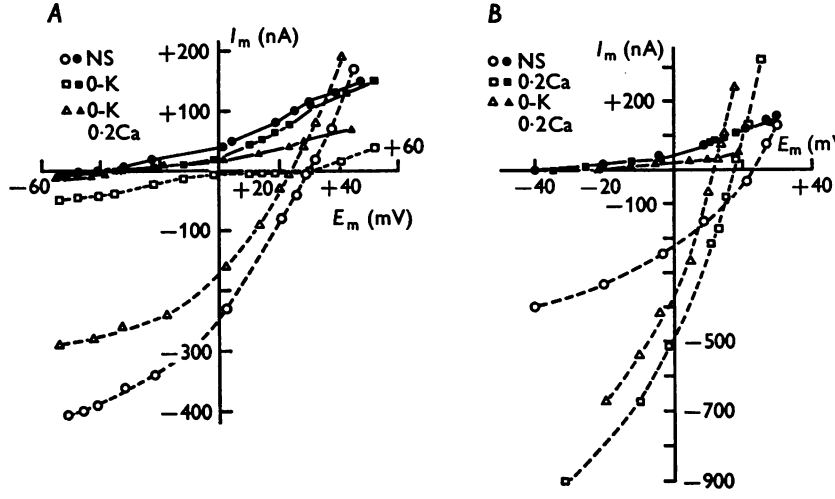

Fig. 7. *A*, current–voltage relations of the membrane in different salines. The sequence was normal barnacle saline (○), 0-K saline (□) followed by 0-K, low Ca^{2+} (0·2 mM) saline (△). Membrane current in the dark was obtained just before the onset of illumination (330 msec) and is represented by the filled symbols; the peak light induced current (total membrane current during illumination minus membrane current in the dark) in each of the salines is represented by open symbols. K^+-free saline depolarized the membrane about 8 mV. The membrane was further depolarized by about 2 mV when Ca^{2+} was reduced. The holding potential was maintained at these levels. *B*, different cell. I–V relations of the membrane in a different sequence of salines: normal barnacle saline, low Ca^{2+} saline, and low Ca^{2+}–0-K saline. In this case, the reversal potential of the light-induced current shifted to a less positive value when K^+ was removed from the saline.

A different sequence of test salines indicated that part of the shift in the reversal potential was due to the reduced Ca^{2+} and part is attributable to reduced K^+ provided that K^+ is reduced in low Ca^{2+}. Fig. 7*B* from a different cell illustrates these changes. Lowering the Ca^{2+} concentration produced little change in the *I–V* relation obtained in darkness as seen by

comparing the data represented by filled circles and squares. However, the LIC was increased twofold and this was associated with linearization of the *I–V* relation; there was a 5 mV negative shift of the potential at which light-induced current reversed sign. A further shift of 5 mV occurred when the K^+ was removed in the low Ca^{2+} saline. The slope of the relation remained about the same as it was in the low Ca^{2+} saline, i.e. the *I–V* relation was shifted along the voltage axis. A change did occur in the *I–V* relation obtained in darkness in the K^+-free, low Ca^{2+} saline. The slope conductance was reduced to about half of what it was in the prior salines. This is the same change that occurred in the K^+-free, low Ca^{2+} saline shown in Fig. 7*A* when the order of the saline changes was different. Thus, in all essential characteristics the *I–V* relations are similar in the final saline, i.e. 0-K, low Ca^{2+}. However, there were differences that depended on whether Ca^{2+} or K^+ was reduced first. There was no apparent shift in the reversal potential of the light-induced current when K^+ was removed before the reduction in Ca^{2+}, yet when Ca^{2+} was reduced after 0-K saline, there was a larger shift in the reversal potential (*ca.* 7–8 mV) than when K^+ was removed after a prior exposure to low Ca^{2+}. Thus, the immediate effect of the K^+-free saline, which is expressed in a reduction of the DRP does not appear to arise because of a large change in the effective e.m.f. of the light-induced current, but from a drastic alteration of the conductance change normally produced by light. However, a sufficiently long exposure to either of these salines results in changes of ion gradients as described in the next section. This is difficult to assess in the K^+-free saline with the voltage-clamp technique, since the light-induced current is abolished.

Intracellular Na^+ and K^+ changes

Intracellular Na^+ and K^+ activities were monitored with ion sensitive micro-electrodes during the same series of solution changes. Changes in the activity of these ions were found that correlate with changes in the *I–V* relations. Cell to cell variation was observed, but generally, an exposure to K^+-free saline produced about a fivefold increase in the internal Na^+ activity (a^i_{Na}) over a period of about an hour, i.e. a^i_{Na} increased from 10 to 50 mM. In separate experiments conducted with the K^+ liquid ion-exchanger micro-electrodes, internal K^+ activity (a^i_K) was decreased by a similar amount in the same time. Reduction of Ca^{2+} in the absence of K^+ greatly facilitated the loss of K^+ and in some cells greatly facilitated the increase in a^i_{Na}. Cells with a lower initial a^i_{Na} (10–20 mM) in normal saline showed somewhat less of a change in 0-K saline or 0-K, low Ca^{2+} saline than cells with a higher resting value of internal Na^+ activity (30–50 mM). The range of Na^+ and K^+ activities obtained from dark-adapted barnacle photoreceptors in this study was the same as that

encountered in other studies with the same type of electrodes (H. M. Brown, unpublished; Brown & Cornwall, 1975).

Fig. 8 shows results from a cell with a measured Na^+ activity (a^i_{Na}) of 13 mM. Light flashes (1 sec) were presented 1/10 sec; the envelope of the potential changes recorded with the Na^+ electrode and the KCl electrode are represented by V_{Na} and E_m respectively. The membrane potential

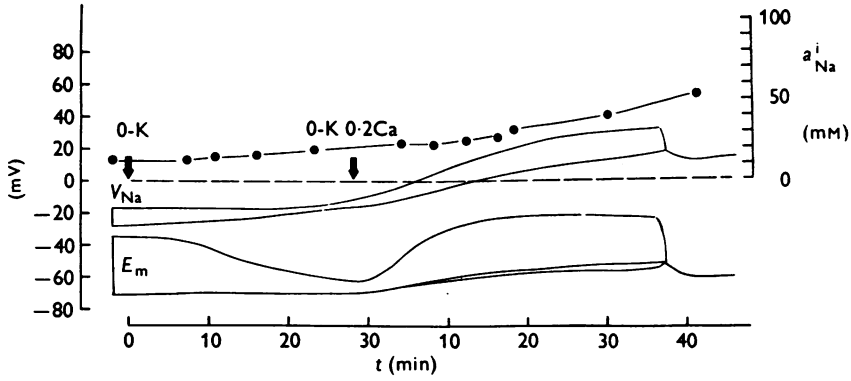

Fig. 8. Changes in internal Na^+ activity in 0-K saline and 0-K, low Ca^{2+} (0·2 mM) saline. Internal Na^+ activity (●) is shown on the right ordinate and the voltage changes of the membrane (E_m) and the ion sensitive electrode (V_{Na}) are represented on the left ordinate. The Figure shows the envelope of the voltage changes traced from the chart record and reduced photographically. Light flashes were presented 1/10 sec and the post-illumination hyperpolarization following each light flash attained − 70 mV. The wedge at the bottom of the E_m trace shows the increase in amplitude of the rapid hyperpolarization in 0-K, 0·2 Ca saline (see Fig. 4). The top of the E_m trace represents the steady phase of the receptor potential. The envelope of the V_{Na} trace is less than the E_m trace because the membrane potential changes to light were attenuated. Since both traces have the same reference potential, an increase in the difference of their base lines reflects an increase in a^i_{Na}.

envelope was traced from the maximum excursion of the post-illumination hyperpolarization to the steady phase of the light response. The response to the membrane potential changes recorded from the Na^+ electrode was attenuated so that the envelope is smaller than that recorded with the voltage electrode. This does not influence measurement of a^i_{Na} since only the difference between the base lines of the V_{Na} and E_m trace are used for this purpose. This difference was used to calculate a^i_{Na} from eqn. (1) and the values are shown plotted (filled circles) in the upper graph. The cell had been penetrated with the Na^+-sensitive pipette and the KCl pipette for about 40 min before the time the changes shown in Fig. 8 occurred. When 0-K saline was applied the depolarizing receptor potential

began to decline slowly for the first 10 min and later more rapidly. This was accompanied by a slow decline in the resting potential of the cell. The base line potential change of the Na^+ electrode was more rapid than the membrane potential change which indicates that a^i_{Na} was increasing as shown in the upper graph. Na^+ activity increased in a fairly linear fashion after removal of K^+ over the first 30 min. During this time the light response was reduced to about 20% of the amplitude in normal saline and Na^+ activity increased from 13 to 22 mM. When Ca^{2+} was reduced one hundredfold in the absence of K^+, a^i_{Na} appeared to increase at a somewhat more rapid rate; 30 min in this saline increased a^i_{Na} from 22 to 41 mM. When Ca^{2+} was reduced, the depolarizing receptor potential recovered and the membrane potential depolarized at a more rapid rate than it had in 0-K saline.

The recovery of internal Na^+ activity in normal barnacle saline is shown in Fig. 9*A*. This cell had been exposed to a 0-K solution for 45 min; before this exposure the resting value of a^i_{Na} in normal saline was 12·5 mM which is similar to that in Fig. 8. The 45 min exposure to K^+-free saline had increased the a^i_{Na} to about 50 mM. Restoration of normal saline in the absence of repetitive light stimulation produced a large hyperpolarization of the membrane (20 mV) which recovered to about the same level as that prior to application of the normal saline. These changes occurred over a time span of about 15 min. Associated with these changes was a gradual decline in the internal Na^+ activity from 50 to about 10 mM. The steady-state level was attained at the same time that the changes in the membrane potential had ceased to occur. In Fig. 9*B* the changes in the membrane potential during the hyperpolarization (●) and decline of the hyperpolarization (○) are shown plotted logarithmically against time. Changes in a^i_{Na} are represented (■). The time course of the membrane hyperpolarization was somewhat less than that from the nadir of the hyperpolarization to the steady-state resting level; $\tau \approx 1$ and 2 min respectively. In some other cells the descent and ascent of the membrane potential changes were more symmetrical. In this particular cell and others examined under the same conditions, changes in the internal Na^+ activity followed a single time course reasonably well. There was quite close agreement in the sum of the time constants of the membrane potential changes and the time constant of the changes in the internal Na^+ activity. Thus, most of the resting membrane potential changes observed to occur under these conditions seem best attributed to K^+-activated electrogenic pump activity (Koike *et al.* 1971).

Removal of K^+ from the external saline produced a reduction in the internal K^+ activity as monitored with a K^+-sensitive liquid ion-exchanger micro-electrode. Data shown in Fig. 10 are from a cell penetrated with

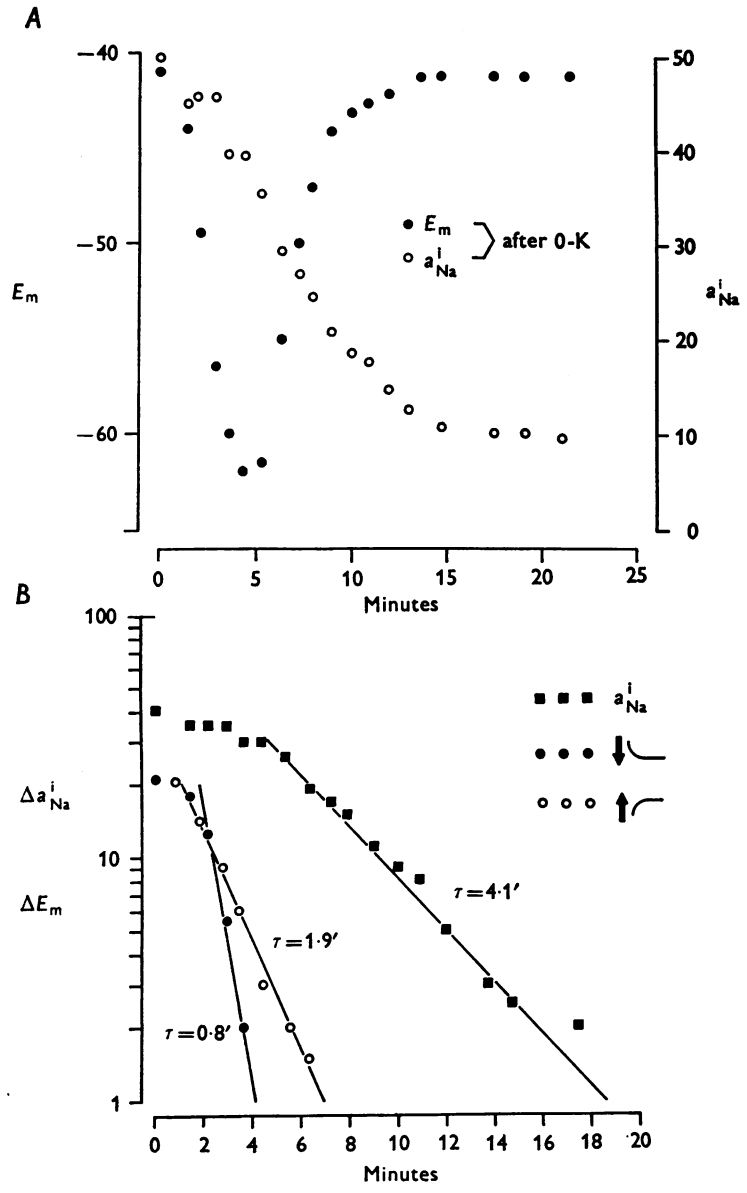

Fig. 9. Recovery following K^+-free saline. *A*, changes of membrane potential (●) and internal Na^+ activity (○) following 45 min in 0-K solution. Different cell from that in Fig. 8. Initial internal Na^+ activity prior to 0-K saline was 12·5 mM. *B*, changes in the membrane potential and a^i_{Na} in NS after a 45 min exposure to 0-K saline; the changes are plotted semilogarithmically against time. The descent of the membrane potential to the maximum negative level is indicated by (●) and the ascent to the steady state is represented by (○). The time constant of the former was about 1 min whereas the latter was about 2 min. Changes of Na^+ activity over the same time period are represented by (■). Internal Na^+ activity changed with a time constant of about 4 min.

KCl and K^+-sensitive pipettes. Almost an hour had elapsed prior to changes in the saline; a steady value of a_K^i = 125 mM had been obtained for about 30 min. When K^+ was removed from the saline bathing the barnacle photoreceptor (0-time) the membrane immediately began to depolarize (filled symbols) and the a_K^i (open symbols) decreased from 125 to 105 mM over a span of 20 min. Reducing Ca^{2+} one hundredfold in the absence of K^+ produced a dramatic decrease in a_K^i and enhanced the rate of membrane depolarization. E_m was reduced by about 10 mV over 30 min in this saline and a_K^i decreased to only 40 mM. This abrupt change

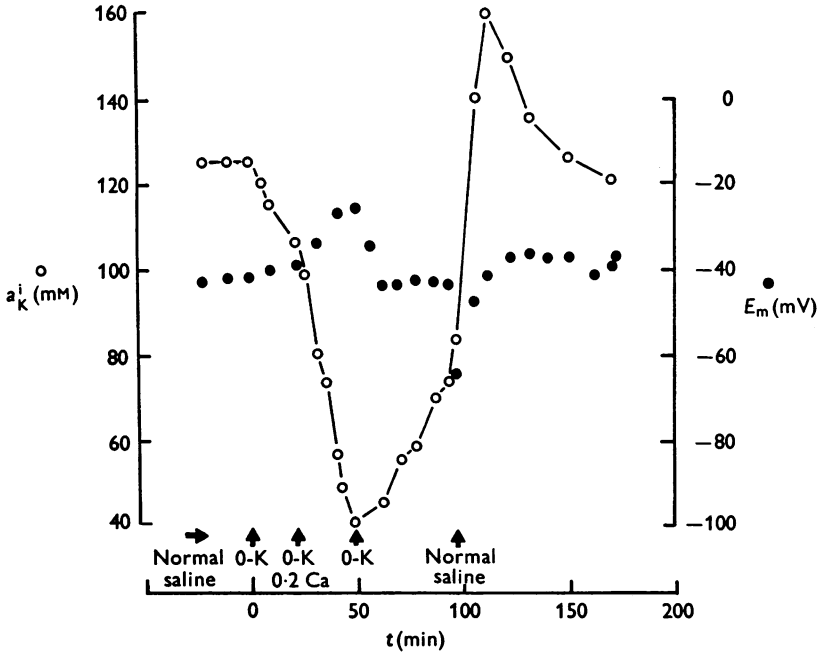

Fig. 10. Measurements of internal K^+ ion activity in a cell exposed to a series of different salines. Internal K^+ activity (○) and membrane potential in darkness (●) in the saline indicated. Internal K^+ activity was about 125 mM after equilibrating for 50 min in normal saline (0 time). Large changes in a_K^i occurred especially when Ca^{2+} was reduced in the absence of K^+. The resting membrane was depolarized significantly in this saline. Restoration of normal barnacle saline restored the a_K^i to a level quite close to the original control value.

in a_K^i was largely due to the reduction of the Ca^{2+} since a_K^i increased from this low level by restoring the Ca^{2+} in the absence of K^+. This manipulation (at t = 50 min) was also accompanied by restoration of the resting membrane potential as previously shown in the cell in Fig. 3*C*. When the full complement of K^+ was restored to the saline the a_K^i

increased as dramatically as it had been reduced in the K^+-free, low Ca^{2+} saline. This was associated with the usual membrane hyperpolarization which in this particular cell exceeded -60 mV and slowly declined to about -40 mV. An abrupt overshoot of a_K^i that exceeded the control level in normal saline had no apparent relation to the slow changes in E_m at this time. Following the overshoot, a_K^i declined slowly over the next 50 min to about 120 mM, close to the previous level in normal saline. The loss of K^+ in this cell cannot be directly equated with the gain in Na^+ shown for the cell in Fig. 8 under similar conditions. In some cells the increase in a_{Na}^i was as great as the reduction in a_K^i shown in the cell in Fig. 10. Unfortunately, it was not possible to monitor a_{Na} and a_K^i simultaneously in the same cell.

K^+ as suppressor of light induced current in low Ca^{2+}

A prolonged exposure to K^+-free saline produced a profound reduction in the light-induced current as shown previously. On the other hand reducing Ca^{2+} can mitigate this effect. In low Ca^{2+} solutions, increasing the K^+ can also reduce this current. This is demonstrated by the experiments summarized in Fig. 11; each symbol represents a different cell. The experiments were conducted as follows. The photoreceptor was exposed to a series of test salines containing increasing amounts of K^+ with a constant background of Na^+ (235 mM) and Ca^{2+}. These salines were obtained by mixing 50 % Na^+ – 50 % Tris saline and 50 % Na^+ – 50 % K^+ saline in different proportions. Each series contained a fixed amount of Ca^{2+}, but Ca^{2+} concentration was varied among different series by substitution with Mg^{2+}. The membrane was depolarized by different amounts in each of the high K^+ salines and when the potential level was stable, the membrane was voltage-clamped to the original value observed in normal saline. The peak light-induced current measured in each of the test salines was normalized to the values of this current in the saline that contained the normal amount of Ca^{2+} (20 mM). The open squares in Fig.11 represent the light-induced current recorded at the same membrane potential in increasing amounts of K^+ (abscissa) at constant Na^+ (50 %) and normal Ca^{2+} concentration (20 mM). Increasing K^+ in the presence of normal Ca^{2+} had no detectable effect on the LIC, nor did increased K^+ affect it in elevated Ca^{2+} (32 mM: △) saline. However, reduction of Ca^{2+} from 20 to 2 mM in normal K^+ greatly augmented the light induced current as shown by filled symbols. Under these conditions, an increase in the external K^+ concentration suppressed the inward light induced current. In two cells (■ and ▲), an increase in K^+ concentration from 15 to 117 mM produced more than a threefold decrease in light-induced current, reducing it to about the value obtained in normal Ca^{2+}. Decreasing

the level of Ca^{2+} to still lower values (1: ○, △ and 0·2 mM: D) produced larger increases in the LIC and this was systematically reduced by increasing the K^+ concentration. The straight lines through the data points represent the best fit determined by a least-squares regression analysis and indicate that the slopes of the relations are reasonably parallel for different Ca^{2+} concentrations below the normal amount. The

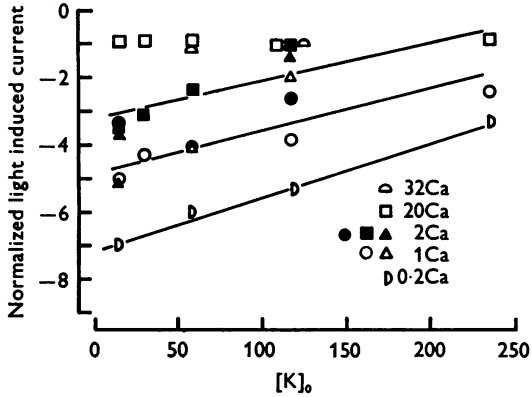

Fig. 11. Light-induced membrane current in different Ca^{2+} concentrations as external K^+ was increased. Each symbol represents a different cell. Light-induced current in different Ca^{2+} concentrations was normalized to the value obtained in a control saline containing 50% Na^+ and normal Ca^{2+}. The membrane was depolarized as the external K^+ concentration was increased; the membrane current was measured at each K^+ concentration by clamping the membrane potential to the value in normal saline (−40 to −50 mV in different cells); thus, all values were obtained at comparable membrane potentials. The preparation was returned to the control saline after each exposure to the higher K^+ concentrations. In normal Ca^{2+} (20 mM) or high Ca^{2+} (32 mM), serial substitution of K^+ for $Tris^+$ ions resulted in little change in inward membrane current. Lowering the Ca^{2+} concentration resulted in larger inward currents and this inward current was reduced as the external K^+ was increased. The straight lines through the data were obtained by a least-squares regression analysis.

exact nature of the relations is uncertain, however, since a case could also be made that the slopes diminish as the Ca^{2+} concentration is decreased. Even though the form of the relations is uncertain, it is clear that K^+ can suppress the light-induced current when augmented by a reduction in the Ca^{2+} concentration and that the amount of suppression is related to the external K^+ concentration. The relations suggested by the regression analysis have some utility since they provide an estimate of the amount of K^+ required to offset the effect of a certain reduced Ca^{2+} concentration. The 2, 1·0 and 0·2 mM-Ca^{2+} curves intersect the high Ca^{2+} curves at 225, 350, and 415 mM-K^+, respectively.

DISCUSSION

Removal of K^+ ions has the same apparent effect on some other receptors as it does on the barnacle photoreceptor. In the frog muscle spindle, Husmark & Ottoson (1971*b*) observed a reduction and final abolition of the receptor potential. Following the return to normal saline after exposure to K^+-free solution, the spindle receptor potential often assumed a rectangular wave form; this was also observed in the barnacle (Fig. 1*A*) and is an effect usually associated with low Ca^{2+} saline as shown in Fig. 3 (also see H. M. Brown *et al.* 1970, 1971). The 'adaptive decline' in the rapidly adapting receptor of the crayfish (Ottoson, 1973) can also be augmented at certain times in 0-K. K^+-free saline has been reported to abolish the depolarizing receptor potential in *Limulus* ventral photoreceptors (Smith, Stell, Brown, Freeman & Murray, 1968) yet an extended exposure to K^+-free saline seems to have little effect on the receptor potential of the honeybee retinula cell (Fulpius & Baumann, 1969; see Discussion).

The dilemma associated with these findings and those presented in the present study is that Na^+ ions represent the major component of the receptor current yet a Na^+-free solution does not completely abolish the receptor potential. On the other hand, K^+-free solutions can abolish the receptor potential entirely. Yet there is little evidence, at least from *Balanus* photoreceptors, that K^+ ions are directly involved in the receptor current (H. M. Brown *et al.* 1970).

The K-induced membrane hyperpolarization observed in the present investigation provides an ostensible explanation of the K^+-free effects on the depolarizing receptor potential. Several lines of evidence indicate that the hyperpolarization is due to active extrusion of Na^+ presumably accumulated during exposure to 0-K^+ saline. It has been shown previously that a long-lasting hyperpolarization can be produced in the barnacle photoreceptor by intracellular injection of Na^+ ions and that the time course of this hyperpolarization and that following a bright light flash are similar (Koike *et al.* 1971). Since light produces a permeability increase of the membrane mainly to Na^+ ions, the mechanism suggested for the membrane hyperpolarization was activation of an electrogenic Na^+ pump. It was shown in this study by direct measurement of a^i_{Na} with Na-sensitive micro-electrodes that Na^+ does accumulate intracellularly during exposure of the photoreceptor to 0-K saline (Figs. 8, 9). Upon return to normal saline there was a large membrane hyperpolarization and a reduction of a^i_{Na}. A K^+-activated hyperpolarization following a directly measured increase in a^i_{Na} has also been reported in snail neurones (Thomas, 1969) and similar phenomena in other preparations is probably due

to the same mechanism (Carpenter & Alving, 1968; Rang & Ritchie, 1968). A reasonable correspondence was found between the sum of the time constants for membrane potential changes and the decrease of a^{i}_{Na} (3–4 min) upon return to normal saline from 0-K saline. Thus, it seems in the barnacle photoreceptor that the K^+-activated hyperpolarization is directly associated with active extrusion of Na^+ that had accumulated in the cell during the exposure to K^+-free solution. An accumulation of Na^+ during K^+-free suggests at least a partial explanation for the decline of the depolarizing receptor potential in the K^+-free saline: loading of the cell with Na^+ with a consequent reduction of the effective e.m.f. of the membrane for the light induced current. It has been shown that reduction of external Na^+ concentration shifts the reversal potential of the light-induced current toward less positive values (H. M. Brown *et al.* 1968, 1970). An increase in the internal Na^+ activity should produce the same effect as a decrease in external Na^+ concentration. Measurements of the changes in internal Na^+ activity during exposure to 0-K^+ saline indicated that the Na^+ gradient was significantly reduced but not by a sufficient amount to account for the large reduction in the depolarizing receptor potential and light-induced current. The maximum increase in a^{i}_{Na} observed in any of the cells studied was from 30 to about 150 mM. Even in this extreme case the Na^+ equilibrium potential would be about +15 mV (a^{o}_{Na} = 315 mM) which still should provide some driving force for Na^+ ions; this could be shown in this cell by inducing a light response in low Ca^{2+} saline, even though the light response was completely abolished in 0-K saline before reduction of Ca^{2+}. In other cells with an initial lower internal Na^+ activity, such as those shown in Fig. 8 or 9, the a^{i}_{Na} only increased to about 50 mM. (E_{Na} = +46 mV) yet the depolarizing receptor potential was still essentially abolished. When external Ca^{2+} was reduced from 20 to 0·2 mM, a^{i}_{Na} increased at an even greater rate yet a depolarizing receptor potential could be elicited that exceeded the membrane potential level in normal saline (Fig. 8). Thus, even though Na^+ accumulation could conceivably account for some reduction in the receptor potential in 0-K, it does not appear to be the primary mechanism since the receptor potential can be restored in 0-K saline if Ca^{2+} is reduced.

An insight into the primary mechanism is gained from reference to the *I–V* relations presented in Fig. 7*A* and *B*. K^+-free, low Ca^{2+} saline can reduce the slope conductance of the membrane in darkness. Consistent with this was the finding with the ion-sensitive electrode that K^+-free saline, especially with low Ca^{2+} can produce a significant reduction in internal K^+ activity (Fig. 10). This implies that the major dark current is carried by K^+ ions and the reduced slope should produce a larger depolarizing receptor potential provided the LIC remains the same. This

was the situation in the low Ca^{2+} saline even though K^+ was absent. This was not the case when K^+ alone was removed. Since there was little evidence of a shift in the effective e.m.f. of the light-induced current in this condition, the major effect of the K^+-free solution is attributable to a reduction of the light sensitive component of membrane conductance. As Fig. 7*A* shows, this change in membrane conductance can be reversed if Ca^{2+} concentration is reduced. The light-induced current returns to values close to or exceeding those in normal barnacle saline under this condition. This suggests that under normal conditions, K^+ ions can antagonize the suppression of Na^+ conductance normally exerted by Ca^{2+} ions and in the absence of K^+, Ca^{2+} can completely shut down the light-induced current. This resembles to some extent the K^+ antagonism of the Ca^{2+} desensitization observed on the end-plate potential (Manthey, 1972). Reducing the Ca^{2+} restores the current despite the fact that a^i_{Na} increases as evidenced by the shift of the 0-current potential in the I–V relations and the Na^+ electrode experiments. A change in the membrane e.m.f. for the light-induced current was not observed in a previous study of Ca^{2+} on this membrane unless Na^+ was removed but the Ca^{2+} was not reduced to as low a level as it was in the present study (H. M. Brown *et al.* 1970).

The sequence in which the test salines were presented affected the relative amounts of light-induced current obtained in Fig. 7*A* and *B*. This probably reflects the effectiveness of the different salines at reducing a^i_{Na}: 0-K, 0·2 Ca > 0-K > 0·2 Ca.

A complement of K^+ ions appears to be essential for maintenance of the normal Na^+ and K^+ concentration gradients; moreover, K^+ ions appear to play a significant but indirect role in generation of the receptor potential, namely K^+ ions maintain the light sensitive component of membrane conductance by antagonizing the suppression of light induced current by Ca^{2+} ions. It was demonstrated previously (H. M. Brown *et al.* 1970) that $\overline{XNa}$, a presumptive Na-carrier or carrier-site complex in barnacle photoreceptor is related to Ca^{2+} concentration by the relation:

$$\frac{Xt}{\overline{XNa}} = 1 + \frac{K_{Na}}{[Na]_0} + \frac{K_{Na}}{K_{Ca}[Na]_0} \cdot [Ca^{2+}]_0. \qquad (2)$$

That is the binding appears to follow the Langmuir isotherm since the relation between the reciprocal light-induced current and Ca^{2+} concentration is a rectangular hyperbola. K^+ appears to antagonize Ca^+ interaction with these sites but the nature of this interaction is not clear from Fig. 11 since the exact form of the relation is uncertain. Additional studies with other multivalent cations are required to reconcile the nature of the mechanism, e.g. screening (D'Arrigo, 1973) or binding. Even though the

exact mechanism is not clear at this point, K^+ does have a complex role in generation of light-induced current. (1) It can antagonize Ca^{2+} suppression of the light-induced current and (2) at high enough concentrations (in low Ca^{2+}) it too can suppress this current. By extrapolating the relations obtained in low Ca^{2+} (Fig. 11) to the level of light-induced current where increased K^+ has little effect (20 and 32 mM-Ca^{2+}), an estimate of the relative efficacy of K^+ and Ca^{2+} at suppressing this current is obtained. From the relation between the concentration of Ca^{2+} and K^+ required to produce the same amount of light-induced current a rate of about 1 mM-Ca^{2+} per 100 mM-K^+ is obtained.

It appears from this study that K^+ and Ca^{2+} play a significant, although indirect role, in determining the wave form of a specific receptor potential and as such provide an ionic basis for some adaptive behaviour that might be common to several different receptors.

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