

# An Electrogenic Sodium Pump in *Limulus* Ventral Photoreceptor Cells

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**ABSTRACT** A hyperpolarization can be recorded intracellularly following either a single bright light stimulus or the intracellular injection of  $\text{Na}^+$ . This after-hyperpolarization is abolished by bathing in  $5 \times 10^{-6}$  M strophanthidin or removal of extracellular  $\text{K}^+$ . Both treatments also lead to a small, rapid depolarization of the dark-adapted cell. When either treatment is prolonged, light responses can still be elicited, although with repetitive stimuli the responses are slowly and progressively diminished in size. The rate of diminution is greater for higher values of  $[\text{Ca}^{++}]_{\text{out}}$ ; with  $[\text{Ca}^{++}]_{\text{out}} = 0.1$  mM, there is almost no progressive diminution of repetitive responses produced by either  $\text{K}^+$ -free seawater or strophanthidin. We propose that an electrogenic  $\text{Na}^+$  pump contributes directly to dark-adapted membrane voltage and also generates the after-hyperpolarizations, but does not directly generate the receptor potential. Inhibition of this pump leads to intracellular accumulation of sodium ions, which in turn leads to an increase in intracellular  $\text{Ca}^{++}$  (provided there is sufficient extracellular  $\text{Ca}^{++}$ ). This increase in intracellular calcium probably accounts for the progressive decrease in the size of the receptor potential seen when the pump is inhibited.

## INTRODUCTION

The mechanism originally proposed for the generation of the receptor potential in *Limulus* photoreception was an increase in membrane conductance, principally to sodium ions (Tomita et al., 1960; Benolken, 1961; Kikuchi et al., 1962). Recent voltage-clamp data obtained for *Limulus* ventral eye photoreceptors support this conductance increase hypothesis (Millecchia and Mauro, 1969 *a, b*; Brown and Mote, 1971).

In this paper, we present evidence that there is an electrogenic sodium pump in ventral eye photoreceptors. Two principal lines of evidence favor the existence of an electrogenic sodium pump in these cells: (*a*) both the response elicited by bright light and the intracellular injection of sodium

ions are followed by marked hyperpolarization of the membrane; and (b) procedures which presumably inhibit the sodium pump rapidly cause the membrane voltage in the dark to become more positive (depolarized). We conclude that the electrogenic pump both contributes directly to "resting" membrane voltage and generates the hyperpolarizations recorded after strong illumination or intracellular injection of sodium ions.

Smith et al. (1968 *b*) proposed that a light-induced change in the activity of an electrogenic sodium pump generated the receptor potential. They demonstrated that several procedures which are thought to inhibit Na-K-ATPase, and hence the sodium pump, caused a partial loss of membrane voltage and a large, progressive decrement in the receptor potential. However, we have shown that in seawater containing a low concentration of calcium ions the activity of the presumed pump can be decreased without causing a decrement of the receptor potential. Our evidence indicates that the pump is not involved directly in the generation of the light response, but rather acts to regulate the internal concentration of sodium ions. We suggest that a rise in the internal  $\text{Na}^+$  concentration has two effects: (a) it stimulates an increase in the activity of a sodium pump; and (b) it leads to an increase in internal  $\text{Ca}^{++}$ , which in turn can attenuate the light-induced conductance changes (Lisman and Brown, 1972).

#### METHODS

The methods for stimulating, intracellular recording, and changing solutions are described in a companion paper (Lisman and Brown, 1972). The composition of the various artificial seawater solutions used in this study are given in Table I. Strophanthidin (Sigma Chemical Co., St. Louis, Mo.) was dissolved in dimethylsulfoxide (DMSO) to make a 0.1 M stock solution. This stock solution was then diluted with the appropriate volume of seawater. Thus, at a concentration of 1 mM strophanthidin, the solution also contained a 1% DMSO concentration. 1% DMSO alone does not produce significant changes in the physiology of the ventral eye photoreceptor cells. The resting potentials of the cells used in this study were  $-45$  to  $-60$  mv, when the cells were dark adapted.

#### RESULTS

##### *The Hyperpolarization Following Bright Illumination*

A dark-adapted photoreceptor cell in the *Limulus* ventral eye responds to bright illumination with a multiphasic receptor potential (Fig. 1 B and Millecchia and Mauro, 1969 *a*). When the illumination is turned off, the membrane voltage becomes markedly more negative (hyperpolarized) relative to the resting voltage preceding illumination (Fig. 1 A). The "after-hyperpolarization" slowly decays back toward the resting potential in the dark. The after-hyperpolarization is larger when preceded by a brighter

TABLE I  
COMPOSITION OF SEAWATER SOLUTIONS

Values are in millimoles per liter.

	NaCl	KCl	CaCl <sub>2</sub>	MgCl <sub>2</sub>	MgSO <sub>4</sub>	NaHCO <sub>3</sub>	Tris-HCl (pH 7.8-8.0)	Sucrose
ASW	423	10	10	22.1	26.2	2.2	15	—
Low-Ca <sup>++</sup> SW	423	10	0.1	22.1	26.2	2.2	15	29.7
K <sup>+</sup> -free SW	423	—	10	22.1	26.2	2.2	15	20.0
K <sup>+</sup> -free, low- Ca <sup>++</sup> SW	423	—	0.1	22.1	26.2	2.2	15	49.7
SO <sub>4</sub> -free SW	368	10	10	48.3	—	—	15	114
High-Ca <sup>++</sup> SW	368	10	50	48.3	—	—	15	—
K <sup>+</sup> -free high- Ca <sup>++</sup> SW	368	—	50	48.3	—	—	15	20

stimulus (Fig. 1 A). Fig. 1 also shows that “spontaneous” fluctuations in membrane voltage occur in the dark; these are the so-called “quantum bumps” (Fuortes and Yeandle, 1964; Millecchia et al., 1966) and are seen in Figs. 1, 3, and 4. Occasionally, a regenerative event arises spontaneously (Dowling, 1968); this happens frequently when the membrane voltage is more hyperpolarized than resting voltage and a positive-going fluctuation occurs (Fig. 1).

The after-hyperpolarization is most often recorded in cells penetrated by a single micropipette. It can be recorded in cells impaled with two electrodes, particularly if the resting voltage in the dark-adapted state occurs on the high resistance portion of the current-voltage relation of the cell membrane (Lisman and Brown, 1971). The hyperpolarization can be recorded following continuous illumination (Fig. 1 A), or can develop during a repetitive sequence of flashes as seen in the first part of the records in Figs. 2 A and 3 A. With repetitive stimuli, the after-hyperpolarization from one stimulus does not dissipate before the next flash occurs; hence, the hyperpolarization tends to accumulate and membrane voltage preceding each stimulus (i.e., the “dark” voltage) can exceed the dark-adapted, resting voltage by 20 mv or more.

If these hyperpolarizations are generated by a sodium pump such as those described in other systems, the hyperpolarizations should be diminished by removal of extracellular K<sup>+</sup> or by cardiac glycosides (Skou, 1965). Fig. 1 A shows that the hyperpolarization subsequent to illumination can be abolished reversibly by removal of the potassium ions from the seawater (K<sup>+</sup>-free SW) bathing the cells. Similarly, the hyperpolarization which accumulates during repetitive illumination can be reversibly removed by briefly bathing the eye in 5 × 10<sup>-6</sup> M strophanthidin (Fig. 2 A) or by briefly perfusing the preparation with K<sup>+</sup>-free SW (Figs. 3 A, 8 B, and 8 C). Also, the

cumulative hyperpolarization does not develop when the cell is illuminated repetitively while bathed in  $K^+$ -free SW (Fig. 3 B). The effects of prolonged treatment with either  $K^+$ -free SW or strophanthidin will be discussed in more detail below.

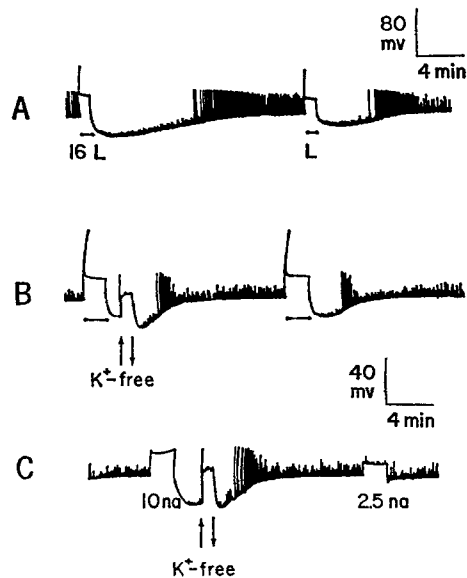
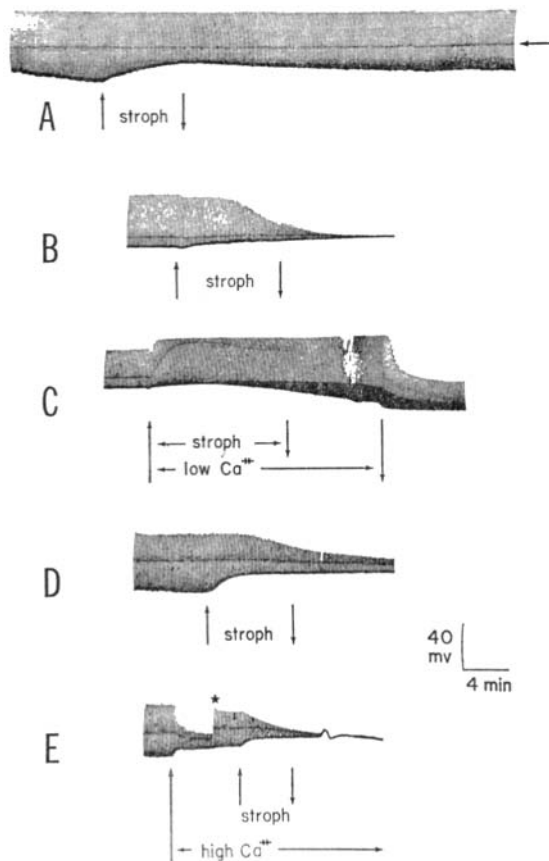


FIGURE 1. Hyperpolarizations recorded intracellularly following both illumination and intracellular iontophoretic injection of  $Na^+$ . Periods of illumination are marked by bars under the voltage traces. So-called "quantum bumps" are seen before stimulation, and recover following illumination; "regenerative" bumps occur as the after-hyperpolarization decays back toward dark, resting voltage. Fig. 1 A, the first 1 min light pulse was 16 times brighter (16L) than the second 1 min pulse (L) and elicited both a larger receptor potential and a larger after-hyperpolarization. Calibration: 80 mv and 4 min. Fig. 1 B, in another cell, when the light-induced after-hyperpolarization was maximum,  $K^+$ -free SW was rapidly perfused through the chamber (first arrow). The membrane quickly depolarized. It repolarized following return of ASW to the chamber (second arrow). There was an additional hyperpolarization following the  $K^+$ -free SW bath. The second light stimulus was identical to the first. Calibration: 40 mv and 4 min. Fig. 1 C, hyperpolarization following intracellular iontophoresis of  $Na^+$  in the dark-adapted cell. The first injection was 10 na for 2 min, the second injection, 2.5 na for 2 min. Following the first injection, a large hyperpolarization developed which was abolished by bathing in  $K^+$ -free SW (first arrow); the hyperpolarization returned when ASW was returned to the chamber (second arrow). Same cell and calibration as (B).

We examined the "voltage dependence" of the depolarization produced by  $K^+$ -free SW in the following way. As Smith et al. (1968) reported, when potassium-free seawater is perfused over a dark-adapted photoreceptor, the membrane depolarizes slightly (Figs. 3 B and 4 A). If the membrane is hyperpolarized by extrinsic current then the depolarization recorded when the cell



**FIGURE 2.** Effects of strophanthidin on membrane voltage and receptor potentials. All repetitive stimuli were 0.5 sec flashes given 1/10 sec. Fig. 2 A, membrane hyperpolarizes during repetitive stimuli. When  $5 \times 10^{-6}$  M strophanthidin in ASW was applied, the membrane began to depolarize. When the strophanthidin was washed out, the membrane began to hyperpolarize. Both during and after treatment with the drug, the sizes of both transient and steady phases of the receptor potential were unaffected. The horizontal arrow marks trace thickening due to the steady phase of the receptor potential. Fig. 2 B, when  $10^{-4}$  M strophanthidin in ASW was applied, the membrane began to depolarize; several minutes later, the receptor potential became decremented. When the drug was washed out, the light response continued to be decremented and recovered partially after prolonged washing with ASW. Fig. 2 C, 90 min after (B).  $10^{-4}$  M strophanthidin in low- $\text{Ca}^{++}$  SW ( $0.1 \text{ mM } \text{Ca}^{++}$ ) was applied for about 10 min. No decrement of the receptor voltage occurred, although the drug was present for a longer time than in (B). The light response was markedly reduced when ASW was returned to the chamber. Fig. 2 D, same as (B) in another preparation. Fig. 2 E, same cell as (D). The cell was bathed in high- $\text{Ca}^{++}$  SW; the membrane depolarized slightly and the light response was decremented. The light intensity was then increased 16 times (star).  $10^{-4}$  M strophanthidin was applied in high- $\text{Ca}^{++}$  SW. The membrane again depolarized rapidly. In addition, the light response to the brighter light began to be decremented more rapidly than the lower extracellular  $\text{Ca}^{++}$  concentrations.

is placed in  $K^+$ -free SW becomes larger. In Fig. 4 B and C, the dark-adapted membrane was hyperpolarized by extrinsic current to the same voltage which occurred during the hyperpolarization following a bright stimulus. The depolarization caused by bathing the dark-adapted cell in  $K^+$ -free SW at this voltage was somewhat larger than at the dark-adapted, resting voltage, but was smaller than that which was caused by the same treatment during the hyperpolarization following illumination by a bright light.

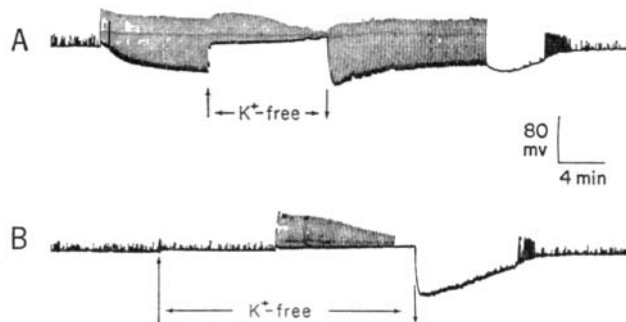


FIGURE 3. Effects of  $K^+$ -free SW on membrane voltage and receptor potentials. So-called "quantum bumps" are seen before stimulation, and recover following illumination; "regenerative" bumps occur as the after-hyperpolarization decays back toward dark, resting voltage. Fig. 3 A, dark-adapted cell stimulated with bright, repetitive flashes (0.5 sec duration, 1/10 sec) initially hyperpolarized. When bathed in  $K^+$ -free SW, the membrane rapidly depolarized; several minutes later, the receptor potential size was reduced. When ASW was returned to the chamber, membrane rapidly hyperpolarized and receptor potential recovered. There was an after-hyperpolarization when repetitive flashes were stopped; membrane voltage then depolarized back to dark-adapted, resting voltage and the "bumps" returned. Fig. 2 B, in the same cell,  $K^+$ -free SW was applied for the time necessary (in A) to reduce significantly the size of the receptor potentials. The membrane depolarized; however, the bumps were not abolished. The responses to flashes (same stimuli as in A) were also not affected until after several minutes of repetitive stimulation. The time-course of the decrement of the light response in (B) (measured from the beginning of the stimuli) was comparable to the time-course of the decrement of the light response in (A), measured from the beginning of the  $K^+$ -free SW bath. Also, there was no hyperpolarization produced during the sequence of flashes in (B) as at the beginning of the trace in (A).

#### *The Hyperpolarization following Sodium Ion Injection*

By impaling a single cell in the ventral eye photoreceptor with two micropipettes, one filled with 3 M KCl and the other with 1 M NaCl, we can simultaneously monitor membrane voltage and iontophoretically inject sodium ions (Lisman and Brown, 1972). After the injection of sodium ions into a dark-adapted cell bathed in artificial seawater (ASW), there is a significant hyperpolarization of the membrane. Such a hyperpolarization is not recorded subsequent to the injection of potassium, lithium, calcium, magnesium, or

chloride ions (Lisman and Brown, 1972, and unpublished data). The hyperpolarization following the injection of sodium ions is larger and more prolonged when greater amounts of  $\text{Na}^+$  are injected. This can be seen either in a dark-adapted cell (Fig. 1 C) or in a cell stimulated by repetitive flashes (Fig. 7 A).

The hyperpolarization subsequent to the iontophoretic injection of sodium ions is similar to the hyperpolarization following a bright illumination. It can be abolished reversibly by rapidly perfusing  $\text{K}^+$ -free SW over the preparation (Figs. 1 C and 5 B). Moreover, the hyperpolarization subsequent to  $\text{Na}^+$  injection does not occur when the preparation is continuously bathed in potassium-free seawater (Fig. 5 A) or in ASW containing  $5 \times 10^{-6}$  M strophanthidin (Fig. 6 B).

In Fig. 7 B, the same size  $\text{Na}^+$  injection evoked a larger after-hyperpolarization when the cell was repetitively stimulated with the brighter of two lights. That is, if the cell is repetitively stimulated with bright flashes, the dark voltage will be more negative than if the cell is stimulated repetitively with dim flashes; when the dark voltage is more negative at the time of the  $\text{Na}^+$  injection, the additional after-hyperpolarization is larger.

#### *Decrement of the Light Response Produced by Presumed Pump Inhibitors*

One procedure which presumably inhibits the activity of the sodium pump in normal cells is the application of a cardiac glycoside (Skou, 1965). As noted above, when we bathed a cell being stimulated by repetitive flashes in seawater containing strophanthidin at a low concentration ( $5 \times 10^{-6}$  M), the membrane almost immediately began to depolarize; when the drug was washed out of the bathing solution, the membrane voltage slowly recovered to its original value. However, neither the voltage at the peak of the transient phase nor the voltage during the steady phase of the light response was affected either during or after the treatment with  $5 \times 10^{-6}$  M strophanthidin (Fig. 2 A).

If a higher concentration of strophanthidin ( $10^{-4}$  M) in the ASW was used (Fig. 2 B), then the membrane began depolarizing rapidly upon application of the drug, and the responses to repetitive stimuli slowly diminished over a period of several minutes, as previously reported by Smith et al. (1968 *b*). The receptor potentials became smaller in a characteristic way: the transient phase was reduced more than the steady phase. When the strophanthidin was removed from the ASW, recovery was very slow and usually incomplete.

As seen in Fig. 2 C, the same eye was bathed in seawater containing 0.1 mM calcium ion (low- $\text{Ca}^{++}$  SW) and  $10^{-4}$  M strophanthidin. The effect of the low- $\text{Ca}^{++}$  SW alone was to increase the sizes of both the transient and steady components of the light response; the receptor potential became almost "square." These effects were first described by Millecchia and Mauro (1969 *a*)

and are examined more fully in a companion paper (Lisman and Brown, 1972). For an incubation time comparable to that shown in Fig. 2 B, the light response was not decremented when the strophanthidin was added to

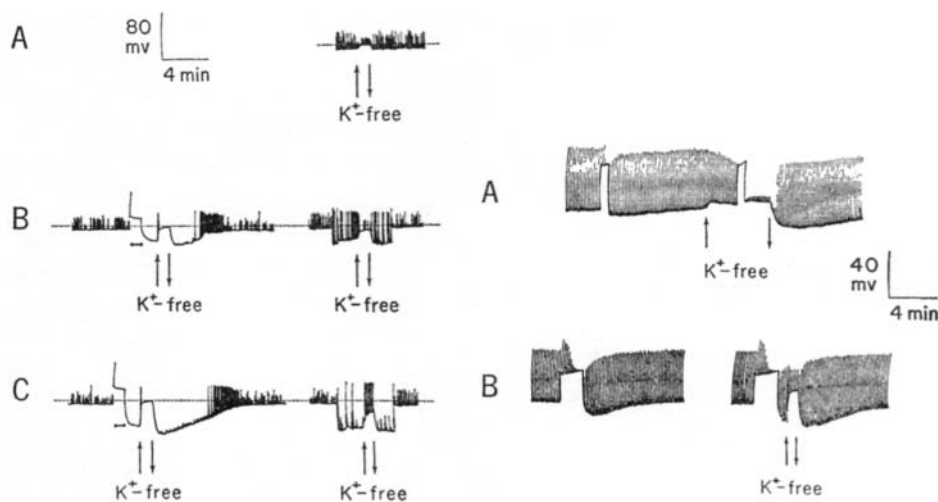


FIGURE 4

FIGURE 5

**FIGURE 4.** The effect of K<sup>+</sup>-free SW during hyperpolarizations following illumination and hyperpolarizations produced by extrinsic current. The absolute level of membrane voltage indicated by the dashed line is the same in all traces. Periods of illumination are marked by bars under the voltage traces. Fig. 4 A, at dark-adapted, resting voltage (note the "bumps"), removal of K<sup>+</sup> from the perfusate leads to a small depolarization. Fig. 4 B, when K<sup>+</sup>-free SW was perfused during a light-induced after-hyperpolarization, the membrane depolarized to the same level as in (A). However, when the cell was hyperpolarized by extrinsic current through the KCl pipette to the same voltage that was reached during the light-induced after-hyperpolarization (the voltage measured by means of a bridge circuit), the depolarization produced by K<sup>+</sup>-free SW was greater than in (A), but did not reach the same voltage as in (A). Fig. 4 C, as in (B), but the stimulating light was 16 times brighter; hence the after-hyperpolarization was greater. Again, with hyperpolarization by extrinsic current, the loss of membrane voltage produced by K<sup>+</sup>-free SW was greater than in (A) or (B), but did not reach the absolute level of membrane voltage in either (A) or (B). See text for explanation.

**FIGURE 5.** The effect of K<sup>+</sup>-free SW on the hyperpolarization following intracellular injection of sodium ions. Repetitive flashes were 0.5 sec in duration, given 1/10 sec. Fig. 5 A, during repetitive flashes, the injection of 10 na through an NaCl-filled pipette produced a small after-hyperpolarization; when the cell was bathed in K<sup>+</sup>-free SW an identical injection of Na<sup>+</sup> produced no after-hyperpolarization. Also, there was very little recovery of the receptor potential following Na<sup>+</sup> injection in this latter case, until the K<sup>+</sup> was returned to the bath (see Lisman and Brown, 1972). Fig. 5 B, in a different cell, the hyperpolarization following the iontophoretic injection of Na<sup>+</sup> is abolished by a brief perfusion of K<sup>+</sup>-free SW. The two injections were identical. In both (A) and (B), the intracellular injection of Na<sup>+</sup> by constant current out of an NaCl-filled pipette produced a progressive reduction of the light response as well as the after-hyperpolarization (see Lisman and Brown, 1972).



low- $\text{Ca}^{++}$  SW. Also, the decrement of the light response did not appear after the strophanthidin was removed from the bath until the calcium ion concentration was returned to normal (Fig. 2 C).

On the other hand, when the calcium ion concentration was greater than normal, application of  $10^{-4}$  M strophanthidin produced a more rapid decre-

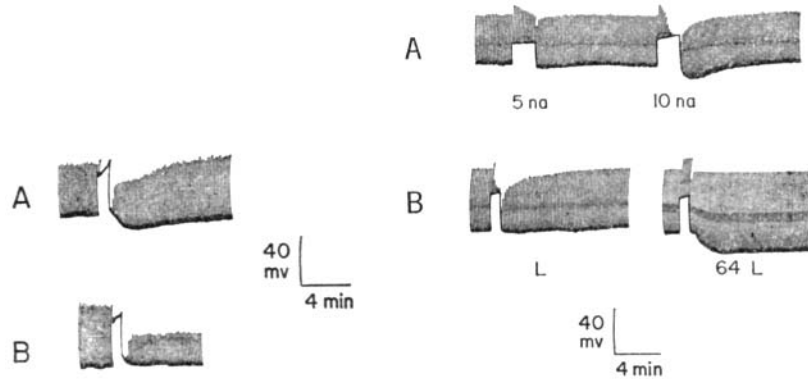


FIGURE 6

FIGURE 7

FIGURE 6. The effect of  $5 \times 10^{-6}$  M strophanthidin on the hyperpolarization following intracellular iontophoretic injection of  $\text{Na}^+$ . Flashes were 100 msec long, given 1/10 sec. Fig. 6 A, during repetitive flashes 15 na was injected through an NaCl-filled pipette. A marked hyperpolarization followed the injection. Fig. 6 B, after several minutes in  $5 \times 10^{-6}$  M strophanthidin ASW, the same injection of  $\text{Na}^+$  was not followed by a hyperpolarization. Also, there was less recovery of the light response following the injection.

FIGURE 7. Effect of light intensity and size of  $\text{Na}^+$  injection on the hyperpolarization following intracellular iontophoretic injection of  $\text{Na}^+$ . Repetitive flashes were 0.5 sec long, given 1/10 sec. Fig. 7 A, the first injection was 5 na, for 2 min, through the NaCl-filled pipette. The second injection was 10 na for 2 min. The after-hyperpolarization (as well as the progressive decrement of the light response) was larger after the larger injection. Fig. 7 B, in another cell, both injections were of 15 na for 60 sec. The light intensity was 64 times greater for the flashes during the second injection. With these brighter stimuli, the membrane was more hyperpolarized before the injection, and the hyperpolarization after the  $\text{Na}^+$  injection was larger. The two records are in register on the voltage axis.

ment of the light response. In Fig. 2 E, the cell was bathed in seawater containing 50 mM  $\text{Ca}^{++}$  and no sulfate ions (high- $\text{Ca}^{++}$  SW). This treatment alone reduced the size of the light response (Lisman and Brown, 1972). We then increased the intensity of the stimulating light (by 16 times) and added  $10^{-4}$  M strophanthidin to the high- $\text{Ca}^{++}$  SW. The membrane began to depolarize rapidly, and in addition, the light response began to decrement quickly. The rate of the decrement of the light response produced by  $10^{-4}$  M strophanthidin in high- $\text{Ca}^{++}$  SW was more rapid than that produced by

$10^{-4}$  M strophanthidin in ASW, although much brighter stimuli were used (Fig. 2 E).

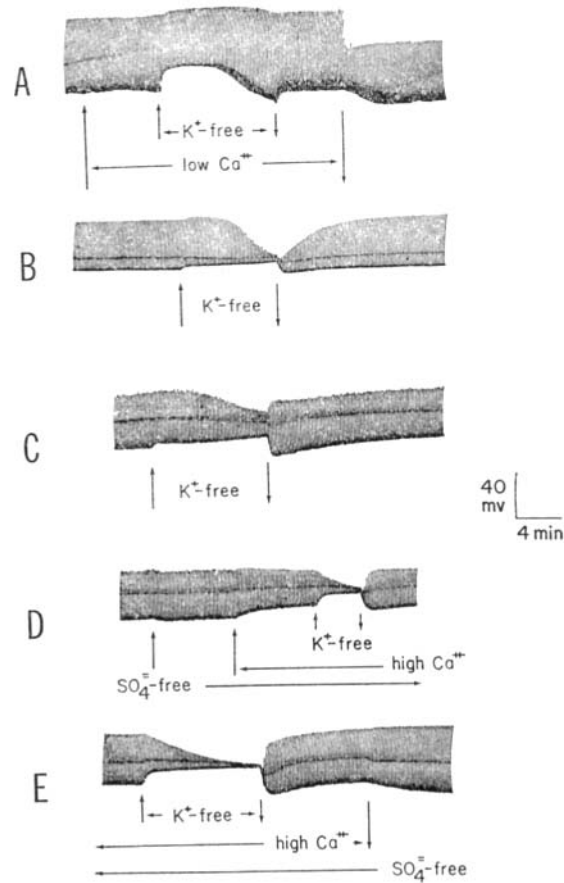
Thus, at low concentrations of strophanthidin in ASW, the membrane rapidly depolarized but the receptor potential was not diminished. At higher concentrations of strophanthidin, the membrane rapidly depolarized and the light response was not (or was very slowly) diminished if there was very little  $\text{Ca}^{++}$  in the seawater bath, was slowly diminished if there was normal  $\text{Ca}^{++}$  in the seawater bath, and was more rapidly diminished with high concentrations of  $\text{Ca}^{++}$  in the seawater perfusate.

Another procedure which presumably inhibits the sodium pump is the removal of potassium ions from the extracellular perfusate. In Figs. 3 A, 8 B, and 8 C, we see the results of such experiments. When we bathed the cell in  $\text{K}^+$ -free SW, the membrane depolarized rapidly and the light response diminished slowly over several minutes. However, if the potassium ions were removed while the cell was bathed in low- $\text{Ca}^{++}$  SW, no decrement in the light response was observed, whereas the depolarization of the membrane still occurred rapidly (Fig. 8 A). Moreover, when the potassium ions were removed while the cell was bathed in high- $\text{Ca}^{++}$  SW, the rate of decrement of the light response was greater than in  $\text{K}^+$ -free SW containing the normal concentration of calcium ions (Fig. 8 D and E).

Light can elicit responses after prolonged incubation in  $\text{K}^+$ -free SW in the dark. In Fig. 3 A, we stimulated the cell with repetitive flashes (1/10 sec); during the repetitive flashes we perfused  $\text{K}^+$ -free SW through the chamber for 10 min. The membrane depolarized in  $\text{K}^+$ -free SW, and remained so until ASW was returned to the chamber. By the end of a 10 min  $\text{K}^+$ -free perfusion, there was a marked decrement of the receptor potentials. After the cell had recovered in ASW, we allowed it to dark adapt, and then perfused with  $\text{K}^+$ -free SW for 10 min in the dark (Fig. 3 B). With the preparation still bathed in  $\text{K}^+$ -free SW, we began to stimulate the cell with the flashes of the same intensity, duration, and repetition rate as in Fig. 3 A. Initially the light responses were approximately as large as those at the beginning of the  $\text{K}^+$ -free SW treatment in Fig. 3 A. The responses again became decremented progressively; the time-course of the diminution of the receptor potentials was comparable to that shown in Fig. 3 A, despite the prolonged bath in  $\text{K}^+$ -free SW which preceded the repetitive stimulation.

#### DISCUSSION

The photoreceptors of the *Limulus* ventral eye produce a hyperpolarization following illumination, similar to that seen in other invertebrate photoreceptors (Benolken, 1961; Koike et al., 1971). Our evidence is consistent with the hypothesis that the hyperpolarization seen subsequent to the end of either a bright stimulus or the iontophoretic injection of sodium ions is produced by



**FIGURE 8.** Effect of changing extracellular  $\text{Ca}^{++}$  on the decrement of the receptor potential produced by  $\text{K}^+$ -free SW. Fig. 8 A, during repetitive stimulation (0.5 sec flashes), low- $\text{Ca}^{++}$  SW (10 mM  $\text{K}^+$ ) was applied. Then,  $\text{K}^+$ -free low- $\text{Ca}^{++}$  SW was perfused through the chamber. The membrane rapidly depolarized, and the responses were not decremented throughout this treatment. When the low- $\text{Ca}^{++}$  SW (10 mM  $\text{K}^+$ ) was returned to the chamber, recovery was rapid. Finally, ASW was again applied. Fig. 8 B, for the same cell as (A), initially ASW was applied. When  $\text{K}^+$ -free SW was perfused through the chamber, membrane voltage again became rapidly more positive; however, the receptor potentials became decremented after several minutes. When ASW was returned, both the receptor potentials and membrane voltage recovered. Fig. 8 C, in another cell, same as (B), for comparison with (D). Fig. 8 D, same cell as (C). Initially,  $\text{SO}_4^{--}$ -free SW replaced the ASW in the chamber. Then the  $\text{Ca}^{++}$  was raised to 50 mM (high- $\text{Ca}^{++}$  SW). The transient phase of the receptor potential became smaller and of shorter duration; the spikelike event became more prominent. When the  $\text{K}^+$  was removed from the high- $\text{Ca}^{++}$  SW, the membrane rapidly depolarized, but the receptor potentials also rapidly began to be decremented. When the  $\text{K}^+$  was returned to the bath, the membrane voltage and receptor potentials recovered. In (E) for the same cell, the intensity of the flashes was raised 16 times while the cell was bathed in high- $\text{Ca}^{++}$  SW. When the  $\text{K}^+$  was removed from the high- $\text{Ca}^{++}$  SW perfusate, the light response still decremented more rapidly than in the ASW control in (C)

the stimulation of an electrogenic sodium pump. The sodium pump in other systems (Kerkut and Thomas, 1965; Thomas, 1969) has been shown to be stimulated by increasing the concentration of intracellular sodium ions. Both the direct iontophoretic injection of  $\text{Na}^+$  and the light response, which is presumably generated by an increase in membrane conductance to  $\text{Na}^+$  (Millecchia and Mauro, 1969 *b*; Lisman and Brown, 1971), ought to increase intracellular  $\text{Na}^+$ ; hence, each procedure could stimulate the putative pump. Incidentally, when the positive current out of the NaCl-filled pipette was adjusted to give a depolarization comparable to that generated by a bright light, the after-hyperpolarizations in each case were approximately the same size (Fig. 1). This is consistent with the idea that  $\text{Na}^+$  carries most of the inward current during the light response. Also, two procedures which inhibit the  $\text{Na}^+$  pump in other systems, namely the removal of extracellular  $\text{K}^+$  or the addition of  $5 \times 10^{-6}$  M strophanthidin (Skou, 1965), reversibly abolish the hyperpolarization following either  $\text{Na}^+$  injection or a bright light stimulus. Both our data and our hypothesis that an electrogenic pump generates the hyperpolarization following illumination agree with the findings of Koike et al. for the barnacle photoreceptor (1971).

Moreover, both the removal of extracellular potassium ions (Fig. 3 B) and the application of cardiac glycosides (Brown and Lisman, unpublished data) usually lead to a depolarization of the membrane of dark-adapted photoreceptor cells. These data are consistent with the hypothesis that an electrogenic pump contributes directly to resting voltage in the dark-adapted cell (Smith et al., 1968 *b*). When the potassium ions are returned to the bath following a  $\text{K}^+$ -free SW incubation, there is a pronounced hyperpolarization to a potential more negative than the normal resting potential. This additional hyperpolarization reflects the increased activity of the pump due to sodium ions which accumulated intracellularly while the pump was inactivated.

In addition, as in some other nerve cells (Carpenter, 1970; Gorman and Marmor, 1970), cooling a dark-adapted photoreceptor cell from above  $20^\circ$  to  $2^\circ\text{C}$  causes the membrane to depolarize.<sup>1</sup> When the cell is stimulated by repetitive bright flashes, the depolarization produced by cooling is greater than when the cell is dark adapted.<sup>1</sup> These results agree with the hypothesis that there is a temperature-dependent electrogenic  $\text{Na}^+$  pump directly contributing to resting voltage in the dark-adapted cell, and that the pump is stimulated (by entry of  $\text{Na}^+$ ) during repetitive flashes to supply additional hyperpolarizing current.

The voltage changes which result from stimulation (Fig. 7 B) or inhibition (Fig. 4) of the presumed pump are voltage dependent. This does not, how-

<sup>1</sup> Holt, C. E., and J. E. Brown. 1972. Ion fluxes in photoreception in *Limulus* ventral eye. I. The potassium light response. *Biochim. Biophys. Acta*. In press.

veer, imply that the pump itself is necessarily voltage dependent. Rather, the pump may be relatively voltage independent, and a large proportion of the voltage dependence of the after-hyperpolarization is due to the interaction of the pump current with the highly nonlinear current-voltage ( $I-V$ ) curve of the cell membrane. This  $I-V$  curve has a low resistance region between resting voltage and 0 v and a high resistance region for voltages more negative than resting voltage (Smith et al., 1968 *a*; Lisman and Brown, 1971). Resting voltage in a dark-adapted cell usually is found to lie in the region of (continuous) transition between high and low resistance. Therefore, we expect that for a given change in pump current, a smaller voltage change will result if the cell is initially at the dark-adapted, resting voltage than if the membrane voltage is initially more negative than the dark-adapted, resting voltage.

Smith et al. (1968 *b*) argued that a change in the activity of an electrogenic sodium pump produced the depolarizing receptor potential. One of their arguments was based on the observation that the light response was progressively attenuated when the photoreceptor was treated so as to inhibit the sodium pump. Figs. 2 B, 3, and 8 B confirm their observations. However, the progressive attenuation of the light response occurs at a much slower rate than does the depolarization of the membrane caused by these treatments. In addition, in contradiction to their hypothesis, we have shown that the light response can be generated even when the putative pump is inhibited. For example, perfusion with seawater containing strophanthidin (Fig. 2 C) or in  $K^+$ -free SW (Fig. 8 A) did not produce attenuation of the light response when the artificial seawater also had a low concentration of calcium ions. Also, receptor potentials could be elicited after prolonged incubation of a dark-adapted photoreceptor in  $K^+$ -free SW (Fig. 3 B), or after cooling a dark-adapted cell to 2°C.<sup>1</sup> Thus, inasmuch as strophanthidin,  $K^+$ -free SW, and cold are presumed to inhibit the activity of the sodium pump, large receptor potentials can be elicited under conditions where the sodium pump is inhibited.

We propose that sodium pump inhibitors produce a gradual attenuation of the receptor potentials during repetitive stimulation, secondarily; that is, inhibiting the sodium pump indirectly leads to an increase in  $[Ca^{++}]_{in}$ . In a companion paper (Lisman and Brown, 1972) we showed that the intracellular injection of  $Ca^{++}$  or  $Na^+$  could attenuate the response to light. This effect of sodium injection (but not that of calcium injection) failed to occur when the extracellular calcium concentration was low. We thus proposed that a rise in intracellular  $Na^+$  can stimulate a rise in the intracellular  $Ca^{++}$ , which in turn attenuates the light response. In this paper, we have shown that the progressive attenuation of the light response caused by application of pump inhibitors does not occur if the extracellular calcium concentration is low. It thus seems likely that the effects of pump inhibition which we observe in normal ASW

are due to a rise in the intracellular  $\text{Ca}^{++}$  which was stimulated by a rise in the intracellular sodium concentration.

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