Voltage-Sensitive Potassium Channels in *Limulus* Ventral Photoreceptors

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**ABSTRACT** The steady-state slope conductance of *Limulus* ventral photoreceptors increases markedly when the membrane is depolarized from rest. The ionic basis of this rectification has been examined with a voltage-clamp technique. Tail currents that occur when membrane potential is repolarized after having been depolarized have been identified. The tail currents reverse direction at a voltage that becomes more positive when $K_o$ is increased. Rectification is reduced by extracellular 4-aminopyridine and by intracellular injection of tetra-ethyl-ammonium (TEA). These results indicate that the membrane rectification around resting potential is due primarily to voltage-sensitive $K^+$ channels. The increase in $g_K$ caused by depolarization is not mediated by a voltage-dependent rise in $Ca^{++}$, since intracellular injection of $Ca^{++}$ causes a decrease rather than an increase in slope conductance. TEA can be used to examine the functional role of the $K^+$ channels because it blocks them without substantially affecting the light-activated $Na^+$ conductance. The effect of TEA on response-intensity curves shows that the $K^+$ channels serve to compress the voltage range of receptor potentials.

**INTRODUCTION**

During the last several years, considerable progress has been made in understanding light-activated membrane conductances in photoreceptors. However, the size and shape of the receptor potential will be influenced not only by the light-activated conductances, but also by voltage-dependent conductances that do not depend directly on light. There is evidence suggesting that voltage-dependent conductances exist in vertebrate rods (Werblin, 1975; Schwartz, 1976), vertebrate cones (Baylor et al., 1974), and invertebrate photoreceptors (Borsellino et al., 1965), but in none of these cases has the ionic basis of the voltage-dependent conductance been established. In *Limulus* ventral photoreceptors, steady-state current-voltage curve shows a region of marked rectification around resting potential (Smith et al., 1968). The results presented here indicate that this rectification is due primarily to voltage-sensitive $K^+$ channels. We have also briefly examined the functional role of these channels and found that they serve to compress the response-intensity curve into a small voltage range. An abstract of our findings has been presented previously (Pepose and Lisman, 1976). Throughout this paper we use the word “channels,” but it
should be made clear at the outset that there is no evidence in *Limulus* favoring channels over other passive permeability mechanisms.

**MATERIALS AND METHODS**

The methods of recording and voltage-clamping were similar to those used previously on the *Limulus* ventral photoreceptor preparation (Lisman and Brown, 1971). The ventral nerve was mounted in a chamber and placed on the stage of a compound microscope. Cells were penetrated with two microelectrodes that approached the cell from opposite sides of the objective. The objective was grounded and served as an electrical shield between the microelectrodes. Clamp current was measured by a current-to-voltage transducer that provided a virtual ground. The output of the transducer had an adjustable single stage RC filter set to 1 ms except where otherwise specified. In most experiments the preparation was continuously superfused at a rate of 5 chamber vol/min. The virtual ground electrode was a chloridized silver wire connected by a 3 M KCl-agar bridge to the chamber at a location downstream from the preparation. The arrangements for light stimulation and measurement were as described previously (Lisman and Brown, 1975a).

Artificial seawater (ASW) was used in all experiments except those in which the reversal potential (V<sub>R</sub>) of tail currents was measured as a function of the extracellular potassium concentration (K<sub>o</sub>). For those experiments a set of solutions was designed in which only the sucrose and potassium concentrations varied (Table I). Superfusate containing 10 mM 4-aminopyridine (Sigma Chemical Co., St. Louis, Mo.) was made by addition of this compound to ASW and adjustment to pH 7.8 with HCl.

Tetra-ethyl-ammonium (TEA) (Eastman Organic Chemicals, Rochester, N. Y.) was injected iontophoretically through microelectrodes filled with 0.1 M TEA. The TEA electrode was used as the voltage-measuring electrode when it was necessary to voltage-clamp cells injected with TEA. In many cases we had difficulty obtaining control responses in cells impaled with a TEA electrode because of TEA leakage. We minimized this problem by recording control responses as rapidly as possible after impalement of the cell. Ca<sup>2+</sup> was injected iontophoretically by the method described by Lisman and Brown (1972a).

For the experiments in which V<sub>R</sub> was measured as a function of K<sub>o</sub>, the protocol was as follows. The reversal potential was initially measured in the 10 mM K<sup>+</sup> ASW. Solutions of elevated K<sup>+</sup> were then presented sequentially, sometimes going from more concentrated to less concentrated, and sometimes vice versa (similar results were obtained with both orders). The change from one solution to the next was done under nonvoltage-clamp conditions. When the voltage in a new solution had stabilized (1-3 min), the membrane was clamped to a preset holding potential and tail currents were measured as described in Results. The measurements of tail currents at different voltages were made at intervals of 10 s or greater. After the measurements in a given solution were completed, the clamp was turned off and the solution changed. At the end of the experiment V<sub>R</sub> was again measured in 10 mM K<sup>+</sup> ASW. Results were reported only if the initial and final values of V<sub>R</sub> differed by less than 3 mV.

**RESULTS**

**Steady-State and Time-Dependent Currents**

Ventral photoreceptors were impaled with two microelectrodes. Steady-state current-voltage curves generated by slow positive-going voltage ramps (10 mV/s) revealed two regions of marked rectification, one in a voltage range more
positive than zero volts, the other near resting potential (Fig. 1) as previously reported (Lisman and Brown, 1971). The slope resistance of the current-voltage curve can be used as a measure of rectification. For the cell illustrated in Fig. 1, the slope resistance in a voltage range (−20 mV to −40 mV) positive of resting potential had a value of 0.9 MΩ. In a voltage range (−60 mV to −100 mV) more negative than resting potential the slope resistance was 29 MΩ (see Figure 1).

### Table 1

VALUES IN MILLIMOLES/LITER

| Solution   | NaCl | KCl | Sucrose | MgCl₂ | MgSO₄ | CaCl₂ | Tris-HCl, pH 7.8 |
|------------|------|-----|---------|-------|-------|-------|------------------|
| ASW        | 424  | 10  | −       | 22    | 26    | 10    | 15               |
| 10 mM K⁺ ASW | 324  | 10  | 150     | 22    | 26    | 10    | 15               |
| 30 mM K⁺ ASW | 324  | 30  | 120     | 22    | 26    | 10    | 15               |
| 50 mM K⁺ ASW | 324  | 50  | 90      | 22    | 26    | 10    | 15               |
| 75 mM K⁺ ASW | 324  | 75  | 52.5    | 22    | 26    | 10    | 15               |
| 100 mM K⁺ ASW | 324  | 100 | −       | 22    | 26    | 10    | 15               |

Osmolarity, 930 mosM.

Inset, Fig. 1). This high-resistance region of the steady-state current-voltage curve will be designated the "leakage resistance." It is the parallel combination of the true leakage resistance and the leakage artificially produced by insertion of the microelectrodes.

The dynamic properties of membrane conductance were examined by giving a series of depolarizing voltage pulses in 10 mV increments. Fig. 2 shows families of depolarizing pulses, each family from a different holding potential. The families of pulses in the lower part of Fig. 2 are similar to those in the...
upper part but were recorded on a faster time scale, at a higher current gain and with less filtering of the current (see caption). At a holding potential of \(-80\) mV or \(-70\) mV, small depolarizing pulses initiated an early inward current. This current accounts for the regenerative spike observed in these cells (Millecchia and Mauro, 1969a) and for the region of negative resistance in current-voltage curves generated with rapidly rising voltage ramps (Lisman and Brown, 1971) in a voltage range near resting potential. The early inward current was apparently inactivated in this cell when the holding voltage was \(-60\) mV or above. Data from several cells indicate that the early inward current was inactivated when the holding voltage was in the range of \(-50\) mV to \(-70\) mV. The outward currents evoked by large depolarizing pulses have several components. In the series in Fig. 2, where holding potential was \(-50\) mV or more, the outward current had an initial transient which decayed to a smaller steady value. If the voltage was stepped to the same potential from less negative holding voltages (\(-40\) mV or more positive), the transient component of the outward current was abolished.

Another component of the time-dependent currents evident in Fig. 2 is the transient current that occurred at the offset of the voltage pulses. The direction of the transient current depended on the holding voltage. These currents are termed "tail currents" (Hodgkin and Huxley, 1952) and are examined systematically in the next section.

**Tail Currents**

The protocol for eliciting tail currents was as follows. The voltage was clamped to a holding potential more negative than resting potential. A depolarizing pulse to 0 mV (\(V_1\)) was applied for a set duration (~1 s), followed by a
hyperpolarizing step to a range of more negative membrane potentials (V$_m$) (Fig. 3 A, C). Associated with the hyperpolarizing step was a brief capacitative current followed by a waning current that declined to a steady or nearly steady value within less than 50 ms. This waning current (between $t_2$ and $t_3$) is the tail current. The direction of the tail current reversed as V$_m$ became more negative (Fig. 3 A, B). A voltage could be found at which there was no tail current. In some preparations a slow upward current drift was observed long after the fast tail current had decayed. Fig. 3 D plots the magnitude of the tail current [$i(t_2)$-$i(t_3)$] vs. the voltage (V$_m$) during the hyperpolarizing step. Points on either side of zero current were connected by eye with a straight line. The value of V$_m$ at which the line intersected the zero current axis was defined as the reversal voltage (V$_R$) of the tail current. Because of the settling time of the voltage clamp, the current during the first 4 ms of the hyperpolarizing step could not be measured. In estimating the initial amplitude of the tail current [$i(t_2)$] we either used the first measurable value (4 ms after the step) or extrapolated the data back to the onset of the step. Fig. 3 D shows that both methods give the same value for V$_R$.

Fig. 4 shows that V$_R$ depended on the duration of the depolarizing pulse ($t_1$), becoming more positive as $t_1$ was increased. Thus, for example, in the cell represented by x symbols in Fig. 4, V$_R$ was $-68$ mV at 0.1 s, $-60$ mV at 1 s, and $-57$ mV at 5.2 s. As a rule, V$_R$ was more negative than resting potential at all times (there was one exception, where at $t_1 = 5$ s, V$_R$ equalled resting potential; see legend to Fig. 4).

Potassium Dependence of Reversal Potential

Photoreceptor cells were superfused with solutions of normal and elevated potassium concentrations. V$_R$ was measured in each solution by the method described in the previous section. The composition of the solutions and the protocol of the experiments are described in Materials and Methods. The data fell into two basic patterns which are shown in Fig. 5 A, B. In Fig. 5 A, V$_R$ became monotonically more positive with increasing external potassium (K$_o$). At high K$_o$, the relationship between V$_R$ and K$_o$ was steep, approximating the slope predicted by the Nernst equation (58 mV for a 10-fold change of K$_o$). At low K$_o$, V$_R$ was relatively insensitive to changes in external potassium. An analysis based on the constant-field equation (Goldman, 1943) has been applied to the data in Fig. 5 A. The constant-field equation states that at room temperature:

$$V_R = \frac{RT}{F} \ln \left[ \frac{(Na_0 + p_rK_0)/(Na_i + p_rK_i)}{(Na_0 + p_rK_0)/(Na_i + p_rK_i)} \right], \quad (1)$$

where $p_r$ is the ratio of potassium permeability to sodium permeability, $p_r/p_{Na}$, for the channels through which the tail currents pass. We have assumed here that the channel is negligibly permeant to divalent cations and to anions. For a different extracellular potassium concentration, K$_o'$,

$$V_R' = \frac{RT}{F} \ln \left[ \frac{(Na_0 + p_rK_0')/(Na_i + p_rK_i)}{(Na_0 + p_rK_0')/(Na_i + p_rK_i)} \right]. \quad (2)$$

We have assumed here that raising K$_o$ has a negligible effect on the intracellular
$\Delta V_R = V'_R - V_R = \frac{RT}{F} \ln \left( \frac{(N_{a_o} + p_r K_o)}{(N_{a_o} + p_r K'_o)} \right)$. (3)

Thus,

$$e^{\Delta V_R / RT} = \frac{(N_{a_o} + p_r K'_o)/(N_{a_o} + p_r K_o)}{\ln \left( (N_{a_o} + p_r K'_o)/(N_{a_o} + p_r K_o) \right)}$$

(4)

The slope ($s$) of the plot of $e^{\Delta V_R / RT}$ vs. $K'_o$ is

$$s = \frac{p_r}{N_{a_o}} + \frac{p_r K_o}{N_{a_o} + p_r K'_o}.$$ (5)

From the data in Fig. 5 A we computed the average change in $V_R$ caused by raising $K_o$ to an elevated level, $K'_o$, and plotted $e^{\Delta V_R / RT}$ vs. $K'_o$ in Fig. 5 C. The points are fairly well fit by a line whose slope, $s$, yields a value of $p_r / p_{na}$ of 27. Thus the data in Fig. 5 A are consistent with the hypothesis that voltage-sensitive potassium channels account for the rectification around resting potential. For the cells shown in Fig. 5 B, $V_R$ became more positive with increasing $K_o$, then decreased, and then increased again. Similar results were obtained in three different preparations. Such results cannot be fit by Eq. (4). We have no ready explanation for these data.

In two experiments we investigated the dependence of $V_R$ on the duration (0.1–5 s) of the depolarizing step in solutions of high-potassium ASW. In ASW, $V_R$ varied with duration by about +10 mV (as in Fig. 4) whereas in 100 mM K $^+$ ASW, $V_R$ varied by only +1 mV. If ventral photoreceptors contain a single class of voltage-sensitive channels, then the time dependence of $V_R$ between 0.1 s and 5 s implies a time-dependent equilibrium potential. On this assumption, the decreased time dependence in 100 mM K ASW suggests that K $^+$ accumulates in the interstitial spaces during a long depolarization, as in squid axons (Frankenhaeuser and Hodgkin, 1956). However, as shown below, the assumption of a single class of voltage-dependent channels is probably not correct for times significantly less than 1 s.

As a check on whether a single class of voltage-sensitive channels generates membrane rectification, we have computed the potassium conductance just

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**Figure 1.** Records of tail currents. A, (Top) Positive voltage pulse brought the voltage to zero followed by a family of hyperpolarizing steps. (Bottom) Simultaneously recorded currents showing inward and outward tail currents during the hyperpolarizing step. B, Tail currents evoked as in A but recorded at a faster time scale. C, Scheme of experiment in A. D, The amplitude of the tail current [$i(t_2) - i(t_3)$] is plotted as function of $V_2$. $x$ represents the amplitudes of the tail estimated by extrapolating the measured current (at 4 ms after the hyperpolarizing step) back to the time of onset of the step. The filled circles are the measured data at 4 ms after the step. The lines have been fit by eye to the data. Both lines cross the zero current axis at the same voltage. This voltage is termed the reversal potential of the tail current ($V_{Rt}$). The data are from the same cell as in Figs. 1 and 2.
before a repolarizing step \([g_K(t_1)]\) and the potassium conductance just after the step \([g_K(t_2)]\). These computations are based on a model, described below, which assumes a single class of voltage-dependent channels. If the model is correct the expected result is that \(g_K(t_1) = g_K(t_2)\) since experiments in other systems show that voltage-sensitive conductances do not change instantaneously when the voltage is altered (Hodgkin and Huxley, 1952).

In our model, delayed outward currents and tail currents are assumed to consist of two parallel components: the current through voltage-sensitive K+ channels, and the current through a leakage conductance \((g_L)\). This model is described by Eq. (6) where \(V_R\) is the zero current (reversal) potential of the channels that carry the delayed outward current and the tail currents, and \(E_L\) is the effective equilibrium potential of the leakage pathway,

\[
i(t) = g_K(V - V_R) + g_L(V - E_L) . \tag{6}
\]

The current \([i(t_1)]\) just before the end of the depolarizing step \((V_1)\) is

\[
i(t_1) = g_K(t_1)(V_1 - V_R) + g_L(V_1 - E_L) . \tag{7}
\]

The current \([i(t_2)]\) just after the cell is hyperpolarized to \(V_2\) is

\[
i(t_2) = g_K(t_2)(V_2 - V_R) + g_L(V_2 - E_L) . \tag{8}
\]

If \(V_2\) is the reversal potential for the tail current, then \(V_2 = V_R\) and

\[
i(t_2) = g_L(V_R - E_L) . \tag{9}
\]
Figure 5. Reversal potential of tail currents \( V_R \) vs. \( K_o \). The results from different cells fell into two basic patterns shown in A and B. The line in A and B is the slope predicted by the Nernst equation. \( V_R \) increased monotonically as \( K_o \) was raised. B, \( V_R \) was not monotonically related to \( K_o \). C, The data in A have been analyzed as described in text. The slope of the line is consistent with potassium channels having a potassium-to-sodium permeability ratio of 27. The theoretical line is a best fit to the data by use of a least-square analysis. \( RT/F \) was 25.2 mV.

From this we compute that

\[
i(t_1) - i(t_2) = \Delta i = g_k(t_1)(V_1 - V_R) + g_l(V_1 - V_R),
\]

or

\[
g_k(t_1) = \frac{\Delta i - g_l(V_1 - V_R)}{V_1 - V_R}.
\]
$\Delta i$ can be measured from data such as in Fig. 3B, and $g_L$ from the high-resistance region (more negative than resting potential) of the steady-state current-voltage curve (such as in Fig. 1). Values of $g_K(t_1)$ are listed in Table II. $g_K(t_2)$ can be calculated from the change in current during the tail (at voltage $V_2$) as the channels go from open (at $t_2$) to completely closed (at $t_3$).

From Eq. (7)

$$
\begin{align*}
\hat{i}(t_2) &= g_K(t_2)(V_2 - V_R) + g_L(V_2 - E_L) \\
\hat{i}(t_3) &= g_K(t_3)(V_2 - V_R) + g_L(V_2 - E_L) \\
\hat{i}(t_2) - \hat{i}(t_3) &= g_K(t_2)(V_2 - V_R) - g_K(t_3)(V_2 - E_K).
\end{align*}
$$

If $V_2$ is sufficiently negative so that in the steady state $g_K(t_3) = 0$ then

$$
\hat{i}(t_2) - \hat{i}(t_3) = g_K(t_2)(V_2 - V_R).$
$$

For the cell in Figs. 1-3, $g_K(t_1)$ ($5.1 \times 10^{-7}$ mho) was nearly equal to $g_K(t_2)$ ($5.3 \times 10^{-7}$ mho). The length of the depolarizing pulse was 1 s. Table II shows that in all cells studied $g_K(t_2)$ was a large fraction of $g_K(t_1)$ (>70%) if the duration of the depolarizing pulse was ~1 s or greater [the combined error in calculating $g_K(t_1)$ and $g_K(t_2)$ is 20-25%; see Table II]. Thus, under these conditions Eq. (6) is approximated, giving credence to the idea that the rectification around resting potential is generated primarily by a single class of voltage-dependent channels.

For very short depolarizing pulses (~100 ms), $g_K(t_2)$ was a small fraction (as low as 43%) of $g_K(t_1)$ (Table II). This suggests that at short times Eq. (6) is invalid, probably because more than one type of voltage-dependent channel is responsible for the currents seen during the first few hundred milliseconds of depolarization.

**Effects of TEA and 4-AP**

TEA blocks K+ channels in a wide variety of preparations. We therefore investigated the effect of TEA on the steady-state current-voltage curve in Limulus. TEA was iontophoretically injected into the cell with a 2-8-nA positive current. During the injection the cell progressively depolarized. The current was periodically reduced so that membrane voltage never became more positive than 0 mV during the injection. Steady-state current-voltage curves before and after TEA injection are shown in Fig. 6. TEA markedly decreased rectification. This observation is consistent with TEA occluding voltage-sensitive potassium channels. Another effect of TEA was to depolarize the cell's resting potential by 0-17 mV (six cells). If voltage-sensitive K+ channels contribute to membrane current at resting potential, then this action of TEA would be expected.

It has been recently reported that voltage-sensitive K+ channels in squid are blocked by extracellular application of 4-aminopyridine (4-AP) (Meves and Pichon, 1975; Yeh et al., 1976). Fig. 7 shows that 10 mM 4-AP applied extracellularly to ventral photoreceptors substantially reduced rectification by decreasing the outward current, again consistent with the notion that rectification is due to K+ channels. In the course of these experiments it was noticed
### TABLE II
COMPARISON OF POTASSIUM CONDUCTANCE JUST BEFORE \( g_{K(t_1)} \) AND JUST AFTER \( g_{K(t_2)} \) REPOLARIZING VOLTAGE STEP

| Experiment date | Duration of depolarizing pulse | \( g_{K(t_1)} \) mho* | \( g_{K(t_2)} \) mho | \( \frac{g_{K(t_2)}}{g_{K(t_1)}} \times 100 \) % |
|-----------------|-------------------------------|------------------------|------------------|----------------------------------|
| 6/20/75         | 60 ms                         | \( 8 \times 10^{-7} \)  | \( 3.5 \times 10^{-7} \) | 43                               |
|                 | 380 ms                        | \( 6.5 \times 10^{-7} \) | \( 4.4 \times 10^{-7} \) | 67                               |
|                 | 560 ms                        | \( 6.2 \times 10^{-7} \) | \( 4 \times 10^{-7} \)  | 65                               |
|                 | 2 s                           | \( 5.5 \times 10^{-7} \) | \( 4.6 \times 10^{-7} \) | 87                               |
|                 | 4.5 s                         | \( 5.4 \times 10^{-7} \) | \( 5 \times 10^{-7} \)  | 93                               |
|                 | 6 s                           | \( 6.7 \times 10^{-7} \) | \( 5.3 \times 10^{-7} \) | 82                               |
| 6/30/75         | 1.3 s                         | \( 5.4 \times 10^{-7} \) | \( 4.25 \times 10^{-7} \) | 80                               |
|                 | 5.6 s                         | \( 5.4 \times 10^{-7} \) | \( 5 \times 10^{-7} \)  | 93                               |
| 7/1/75          | 110 ms                        | \( 9.3 \times 10^{-7} \) | \( 4.5 \times 10^{-7} \) | 48                               |
|                 | 1.1 s                         | \( 5.2 \times 10^{-7} \) | \( 4.5 \times 10^{-7} \) | 87                               |
|                 | 5.2 s                         | \( 4.3 \times 10^{-7} \) | \( 3 \times 10^{-7} \)  | 70                               |
| 7/10/75         | 100 ms                        | \( 9.9 \times 10^{-7} \) | \( 5.6 \times 10^{-7} \) | 57                               |
|                 | 1.2 s                         | \( 7.7 \times 10^{-7} \) | \( 5.3 \times 10^{-7} \) | 70                               |
|                 | 5.2 s                         | \( 7.2 \times 10^{-7} \) | \( 7.6 \times 10^{-7} \) | 106                              |
| 7/11/75         | 130 ms                        | \( 5.3 \times 10^{-7} \) | \( 3.8 \times 10^{-7} \) | 72                               |
|                 | 1.2 s                         | \( 5.4 \times 10^{-7} \) | \( 2.6 \times 10^{-7} \) | 76                               |
|                 | 5.2 s                         | \( 3.1 \times 10^{-7} \) | \( 3.3 \times 10^{-7} \) | 106                              |

* The estimated error in determining \( g_{K(t_1)} \) is 5%.

† \( g_{K(t_2)} \) was measured from the amplitude of tail currents at a voltage slightly positive (~10 mV) of \( V_R \). At such voltages the time course of the tail is slower than at more negative voltages (Fig. 3 A, B), thus minimizing the error that occurs in extrapolating the measured tail current to the onset of the hyperpolarizing step. Nevertheless, the error could be as large as 10%. An additional 10% error is introduced in calculating \( (V_T - E_{K}) \) since the error in measuring \( E_{K} \) is approximately 1 mV. Thus \( g_{K(t_2)} \) may be in error by 20%.

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**Figure 6.** Current-voltage curves recorded under voltage clamp before and after iontophoretic injection of TEA \((1.4 \times 10^{-6} \text{ C})\).
that the spontaneous discrete waves (Adolph, 1964) disappeared when 4-AP was applied, and reappeared when 4-AP was removed. Light-evoked responses were initially abolished by 4-AP, but there was a partial recovery of amplitude after about 10 min in 4-AP.

In contrast to 4-AP, TEA did not abolish spontaneous discrete waves nor did it substantially alter the responses to dim flashes. This encouraged us to study the functional role of the voltage-sensitive channels since it seemed likely that we could block the voltage-sensitive K⁺ conductance using TEA without altering light-activated conductances. The results of such experiments are described in the next section.

![Figure 7](image.png)

**Figure 7.** Current-voltage curves recorded before and 5 min after application of 10 mM 4-aminopyridine. The effect was reversible.

**Effects of TEA on Receptor Potentials**

The contribution of voltage-sensitive K⁺ channels towards shaping the receptor potential was studied by measuring light-induced current and voltage responses before and after intracellular injection of TEA. The plateau potential after TEA injection was much larger than before injection, but the underlying sodium conductance was not increased (Fig. 8A). Therefore, the increase in the amplitude of the plateau after TEA injection was due not to an increase in the light-induced sodium conductance, but rather to the partial occlusion of the voltage-sensitive K⁺ channels which would normally be open during the plateau response to bright illumination. Complete plateau-response vs. intensity curves were recorded before and after TEA injection. The response-intensity
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**Figure 8.** Effect of intracellular TEA on receptor potentials. A, The upper traces show receptor potentials evoked by a 530-nm stimulus ($2 \times 10^{-5}$ W/cm$^2$) before and after intracellular TEA injection ($7 \times 10^{-7}$ C). The lower traces show the light-induced currents measured at resting potential before and after TEA injection. B, Steady-state response vs. light intensity measured before and after TEA injection. The unattenuated beam had an intensity of $2 \times 10^{-4}$ W/cm$^2$. Responses were evoked by starting with the dimmest stimuli, with about 30 s between successive stimuli.

Curve was graded over a much wider voltage range after injection than before injection (Fig. 8 B).

**Effect of Ca$^+$ on Current-Voltage Curves**

Meech (1974) has shown that increasing Ca$^+$ can increase $g_K$ in some molluskan neurons. It therefore seemed possible that the increase in $g_K$ that occurs when ventral photoreceptors are depolarized arises as a secondary consequence of a
voltage-dependent increase in $C_a$. To test this hypothesis we injected $Ca^{++}$ ions into the cytoplasm and examined the effect on the steady-state current-voltage curve. Fig. 9 A shows that $Ca^{++}$ injection had relatively little effect on the current-voltage curve at voltages more negative than resting potential; as the voltage was made more positive than resting potential there was a progressive attenuation of the normal outward current, and a decrease in slope conductance. These effects were completely reversible within 10 min after the injection. A quantitative examination of this effect is not possible since $Ca^{++}$ injected by iontophoresis does not spread uniformly around the cell (Fein and Lisman, 1975). However, the data do show conclusively that the increase in $g_K$ produced by depolarization cannot arise as a secondary consequence of an increase in $C_a$. This conclusion is further strengthened by the observation that rectification around resting potential is not affected by superfusion with 1%

Figure 9. The effect of intracellular $Ca^{++}$ injection on the current-voltage curves and the comparison of this effect to that of light adaptation. A, Current-voltage curves recorded before and 10 s after a $Ca^{++}$ injection (6.6 nA pulses 0.4 s in duration were applied at a rate of 1/s for 5 min). The injection had little effect on resting potential as reported by Lisman and Brown (1972a, b). Injection caused a decrease in slope conductance at voltages more positive than rest. B, Current-voltage curves recorded before and 10 s after a 10-s light ($10^{-4}$ W/cm$^2$). The effect of light is qualitatively similar to that of $Ca^{++}$. Because the effects of both light and $Ca^{++}$ are graded, it would have been possible to produce effects which were quantitatively more similar.
Ca$^{++}$ seawater (not shown). Fig. 9 B shows a current-voltage curve measured in a dark-adapted cell and then measured shortly after a bright light is turned off. The after-effect of light is to cause a voltage-dependent decrease in slope conductance, as reported previously by Lisman and Brown (1971). The similarity of Fig. 9 A and B will be dealt with in the Discussion.

**DISCUSSION**

**V-Sensitive K$^+$ Channels Underlie the Steady-State Current-Voltage Curve**

Two lines of evidence indicate that the rectification of the steady-state current-voltage curve around resting potential is due primarily to voltage-sensitive potassium channels. The first line of evidence comes from the study of tail currents. These currents have a reversal potential that is dependent on extracellular potassium in a manner consistent with a voltage-sensitive channel having a high potassium-to-sodium permeability ratio (Fig. 5A, C). The second line of evidence is pharmacological. TEA, which blocks K$^+$ channels in several systems (Armstrong and Binstock, 1965; Armstrong and Hille, 1972), and 4-amino-pyridine, which blocks K$^+$ channels in squid axons (Meves and Pichon, 1975; Yeh et al., 1976), both substantially reduce the rectification of *Limulus* ventral photoreceptors (Figs. 6, 7).

The presence of voltage-sensitive K$^+$ channels in *Limulus* helps to explain several previously reported results. Holt and Brown (1972) measured radioactive K$^+$ efflux from *Limulus* ventral eyes and observed a light-induced increase in the rate of efflux. Analyzing their data by using the Goldman (1943) equation, they concluded that the light-induced K$^+$ efflux was larger than expected if $g_K$ was independent of light and voltage. Our results indicate that $g_K$ is voltage-dependent, suggesting that a large fraction of the light-induced K$^+$ efflux occurs because light depolarizes the cell and thereby increases $g_K$. A part of the increase in K$^+$ efflux may also be due to the potassium permeability of the channels directly opened by light (Brown and Mote, 1974). Millecchia and Mauro (1969b) reported that in 350 mM K$^+$, the current-voltage curves in ventral photoreceptors show a negative resistance region in the voltage range between $-20$ and $-40$ mV. A negative resistance region of this sort is expected if $E_K$ is close to zero and the principal membrane conductance is a potassium conductance that decreases as membrane potential is hyperpolarized from zero (Meech and Brown, 1976).

A possible mechanism for the increase in $g_K$ produced by depolarization is that depolarization increases Ca$_i$, and Ca$_i$ increases $g_K$, as has been found in neurons of the esophageal ganglion of the mollusk *Helix pomatia* (Meech, 1974; Meech and Standen 1975). We have shown that this is not the principal mechanism operating in *Limulus*, since increasing Ca$_i$ by iontophoresis causes a decrease in slope conductance (Fig. 9 A). Furthermore, the rectification around rest is not reduced by removing extracellular Ca$^{++}$. It therefore seems likely that the rectification around resting potential in *Limulus* is due primarily to K$^+$ channels which are directly voltage-sensitive.

The decrease in slope conductance produced by Ca$^{++}$ injection was somewhat
unexpected. Only recently has a similar effect been reported: Heyer and Lux (1976) observed that Ca++ injection decreased the voltage-sensitive K+ conductance in bursting pacemaker neurons of the mollusk *Helix pomatia*. The effect of Ca++ injection in *Limulus* is highly voltage-dependent, being negligible at voltages more negative than resting potential and increasing as the membrane is depolarized from rest (Fig. 9A). This voltage-dependence resembles that of the voltage-sensitive K+ conductance. It therefore seems plausible that raised Ca_i produces a decrease in slope conductance in the ventral photoreceptor by decreasing the voltage-sensitive K+ conductance; however, alternative mechanisms by which Ca_i might affect the dark conductance cannot be ruled out at this time.

Modulation of the dark conductance by Ca_i may occur during normal functioning of *Limulus* photoreceptors. Lisman and Brown (1971) showed that in addition to the light-activated sodium conductance, there is a second light-dependent membrane process (the "slow process") which responds much more sluggishly to changes in illumination. The ionic mechanism of the slow process has not been identified but it is clearly different from that of the sodium conductance change. As illustrated in Fig. 9B, the slow process has a strong voltage dependence that resembles the effect of Ca++ injection. A unifying hypothesis is that the light-induced increase in Ca_i, demonstrated by Brown and Blinks (1974), affects the dark conductance, thereby generating the slow process. This hypothesis leads to the prediction that the slow process should be blocked by the intracellular injection of EGTA, which blocks the light-induced rise in Ca_i (Brown and Blinks, 1974; Lisman and Brown, 1975b).

In this paper we have not dealt with the negative resistance region of the steady-state current-voltage curve (Fig. 1); however, one comment about the underlying mechanisms seems warranted. Meech and Brown (1976) have pointed out that a very similar negative resistance region occurs in *Limulus* and *Helix* neurons and suggested that both have the following explanation. Small or moderate depolarizations lead to the opening of Ca++ channels and an influx of Ca++. The consequent increase in Ca_i opens Ca++-sensitive K+ channels. If, however, the depolarization is large enough to approach the Ca++ equilibrium potential, the rise in Ca_i will be small and the resulting rise in g_K will be small. The negative resistance region occurs because g_K decreases with increasing depolarization. This explanation seems unsuitable for *Limulus* since raised Ca_i appears to close K+ channels rather than open them. A more plausible hypothesis is that depolarization into the voltage range of the negative resistance region leads to a large increase in Ca_i, which in turn attenuates the K+ currents.

**Complexity of Transient Events**

Whereas the steady-state current-voltage curves can be accounted for largely in terms of a single class of voltage-sensitive K+ channels, the transient events that occur when the membrane is rapidly depolarized are considerably more complex. The first result of rapid depolarization is an inward current of unknown ionic mechanism (Fig. 2). It is unclear how fast the inward current decays and thus to what extent it overlaps with the later outward currents. The
functional role of the inward current is to amplify the voltage fluctuations produced by very dim stimuli (Dowling, 1968). The inward current is followed by an outward current. For large depolarizations the outward current has an initial transient that decays to a steady outward current in about 1 s. The transient component of the outward current is inactivated at holding potentials just positive of resting potential, a voltage range where the steady-state outward current is only partially activated. In these respects the outward currents in *Limulus* resemble those of the transient and steady-state outward currents in molluskan neurons (Connor and Stevens, 1971) which arise from pharmacologically separable K⁺ channels (Neher and Lux, 1972). The transient currents in *Limulus* may thus be the sum of currents through three different channels: two types of K⁺ channels and a channel of unknown ion selectivity that gives rise to the early inward current.

Complicating the time-dependent events still further is the possibility that the extracellular potassium concentration rises during a long depolarization. This is the simplest interpretation of the observation that the reversal potential of the tail current becomes more positive as the duration of depolarization is increased (Fig. 4). In support of this interpretation is the observation that the time dependence of $V_R$ is greatly reduced in high K⁺ seawater. Measurements of $K_o$ in the extracellular space around honeybee photoreceptors indicate that $K_o$ rises during illumination (Tsacopoulos and Coles, 1977). Recordings of a slow light-induced depolarization in nearby glia also suggest a rise in $K_o$ (Bauman, 1975; Bertrand, 1974). Changes in $K_o$ may well explain, in part, the time dependence of $V_R$ in *Limulus*, but the full explanation is likely to be more complex. The calculations of $g_K$ just before and after repolarization (Table II) suggest that the early outward currents are due to more than one type of channel. Thus changes in the relative contributions of different types of channels may also contribute to the changes in $V_R$.

**Functional Significance of V-Sensitive K⁺ Channels**

Photoreceptors are nonlinear transducers: they convert a large range of light intensities into a small range of steady-state voltages (the amplitude of the maintained receptor potential varies roughly as the logarithm of light intensity). This transduction can be considered to occur in two stages. The first involves the linkage between isomerization of rhodopsin and the change in membrane permeability, which in *Limulus* ventral photoreceptors is an increase in conductance, primarily to sodium (Millecchia and Mauro, 1969b; Brown and Mote, 1974). In the second stage, the increase in sodium conductance produces a depolarization that depends quantitatively on the magnitude of the dark conductance. It has previously been shown (Lisman and Brown, 1975a) that the first stage of transduction is highly nonlinear: the steady-state sodium conductance per absorbed photon is orders of magnitude smaller at high intensities than at low intensities. This regulation is probably mediated by a light-induced rise in $C_a$ (Lisman and Brown, 1972a, b; Brown and Blinks, 1974; Lisman and Brown, 1975b). Our results show that the second stage of transduction introduces further nonlinearity. The small increase in sodium
conductance produced by a dim light is efficient in causing depolarization because the conductance of the dark membrane is low. The larger change in sodium conductance produced by a brighter light is less efficient in causing depolarization because depolarization is counteracted by the opening of voltage-sensitive K⁺ channels. If these channels are blocked, the plateau depolarization is increased, and the response intensity curve is graded over a larger range of membrane potential (Fig. 8B). Thus one role of the K⁺ channels is to compress the response-intensity curve into a small voltage range.

Voltage-sensitive K⁺ channels also influence the response to incremental flashes and thus affect the increment-threshold curves, but in this context the role of K⁺ channels is complex and unclear. Insofar as K⁺ channels cause an increase in the cell's load conductance during the response to a background light, the voltage change produced by an incremental change in $g_{Na}$ will be reduced. However, insofar as the K⁺ channels decrease the depolarization produced by a background light, the electrochemical gradient for sodium will be large and the voltage change produced by an incremental change in $g_{Na}$ will be increased. Further complexity arises from the fact that the change in $g_{Na}$ produced by a flash of given intensity depends on voltage (Millecchia and Mauro, 1969b) and therefore will be influenced by the presence of K⁺ channels.

The most dramatic effect of blocking K⁺ channels with TEA (Fig. 8A) is to nearly equalize the amplitude of the transient and plateau phases of the receptor potential. A similar alteration of response waveform is seen after intracellular injection of the calcium buffer, EGTA (Lisman and Brown, 1975b). It may be instructive to consider the entirely different mechanisms by which these drugs produce similar alterations of the receptor potential. When a long bright stimulus is turned on, there is a large increase in $g_{Na}$ which then declines dramatically to a smaller plateau level. Voltage-clamp studies indicate that this decline is blocked or reduced by intracellular EGTA (Lisman and Brown, 1975b). Under these conditions the maintained value of $g_{Na}$ is very large and is sufficient to depolarize the cell to nearly the level of the transient even though the K⁺ channels are functional, as indicated by normal rectification around resting potential after EGTA injection (Lisman and Brown, 1975b). In contrast, TEA injection blocks the K⁺ channels but has no substantial affect on the kinetics of $g_{Na}$. Under these conditions the load conductance is so low that the small plateau value of $g_{Na}$ is sufficient to produce a depolarization nearly equal to that of the transient. These examples illustrate that both voltage-dependent changes in $g_{k}$ and time-dependent change in $g_{Na}$ are important determinants of response waveform.

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REFERENCES

ADOLPH, A. 1964. Spontaneous slow potential fluctuations in the Limulus photoreceptor. J. Gen. Physiol. 48:297–322.

ARMSTRONG, C. M., and L. BINSTOCK. 1965. Anomalous rectification in the squid giant axon injected with tetraethylammonium chloride. J. Gen. Physiol. 48:859–872.

ARMSTRONG, C. M., and B. HILLE. 1972. The inner quaternary ammonium ion receptor in potassium channels of the node of Ranvier. J. Gen. Physiol. 59:388–400.

BAUMANN, G. 1975. Electrophysiological properties of the honey bee retina. In The Compound Eye and Vision of Insects. G. A. Horridge, editor. Clarendon Press, Oxford. 53–74.

BAYLOR, D. A., A. L. HODGKIN, and T. D. LAMB. 1974. The electrical response of turtle cones to flashes and steps of light. J. Physiol. (Lond.). 242:685–727.

BERTRAND, D. 1974. Etude des propriétés electrophysiologiques des cellules pigmentaires de la retina du Faux-Bourdou. Ph.D. Thesis. University of Geneva. 1650.

BORSELLINO, A., M. G. F. FUORTES, and T. G. SMITH. 1965. Visual responses in Limulus. Cold Spring Harbor Symp. Quant. Biol. 30:429–443.

BROWN, J. E., and J. R. BLINKS. 1974. Changes in intracellular free calcium concentration during illumination of invertebrate photoreceptors: detection with aequorin. J. Gen. Physiol. 64:643–665.

BROWN, J. E., and M. I. MOTE. 1974. Ionic dependence of reversal voltage of the light response in Limulus ventral photoreceptors. J. Gen. Physiol. 63:337–350.

CONNOR, J. A., and C. F. STEVENS. 1971. Voltage clamp studies of a transient outward membrane current in gastropod neural somata. J. Physiol. (Lond.). 215:21–30.

DOWLING, J. E. 1968. Discrete potentials in the dark-adapted eye of the crab Limulus. Nature (Lond.). 217:28–31.

FEIN, A., and J. LISMAN. 1975. Localized desensitization of Limulus photoreceptors produced by light or intracellular calcium ion injection. Science (Wash. D.C.). 187:1094–1096.

FRANKENHAUSER, B., and A. L. HODGKIN. 1956. The after-effects of impulses in the giant nerve fibres of Loligo. J. Physiol. (Lond.). 131:341–376.

GOLDMAN, D. E. 1943. Potential impedance and rectification in membranes. J. Gen. Physiol. 27:37–60.

HODGKIN, A. L., and A. F. HUXLEY. 1952. Currents carried by sodium and potassium ions through the membrane of the giant axon of Loligo. J. Physiol. (Lond.). 116:449–472.

HEYER, C. B., and H. D. LUX. 1976. Control of the delayed outward potassium currents in bursting pacemaker neurones of the snail, Helix pomatia. J. Physiol. (Lond.). 262:349–382.

HOLT, C. E., and J. E. BROWN. 1972. Ion fluxes in photoreception in Limulus polyphemus ventral eye. I. The response of potassium efflux to light. Biochim. Biophys. Acta. 274:140–157.

LISMAN, J. E. 1971. An electrophysiological investigation of the ventral eye of the horseshoe crab Limulus polyphemus. Ph.D. Thesis. M.I.T., Cambridge, Mass.

LISMAN, J. E., and J. E. BROWN. 1971. Two light-induced processes in the photoreceptor cells of Limulus ventral eye. J. Gen. Physiol. 58:544–561.

LISMAN, J. E., and J. E. BROWN. 1972a. The effects of intracellular iontophoretic injection of calcium and sodium ions on the light response of Limulus ventral photoreceptors. J. Gen. Physiol. 59:701–719.
Lisman, J. E., and J. E. Brown. 1972b. The effects of intracellular Ca\(^{2+}\) on the light response and on light adaptation in *Limulus* ventral photoreceptors. In The Visual System: Neurophysiology, Biophysics and their Clinical Applications. G. B. Arden, editor. Plenum Publishing Co., New York. 29–33.

Lisman, J. E., and J. E. Brown. 1975a. Light-induced changes of sensitivity in *Limulus* ventral photoreceptors. *J. Gen. Physiol.* 66:473–488.

Lisman, J. E., and J. E. Brown. 1975b. Effects of intracellular injection of calcium buffers on light adaptation in *Limulus* ventral photoreceptors. *J. Gen. Physiol.* 66:489–506.

Meech, R. W. 1974. The sensitivity of *Helix aspersa* neurones to injected calcium ions. *J. Physiol.* (Lond.). 237:259–277.

Meech, R. W., and H. M. Brown. 1976. Invertebrate photoreceptors: a survey of recent experiments on photoreceptors of *Balanus* and *Limulus*. *Perspect. Exp. Biol.* 1:331–351.

Meech, R. W., and N. B. Standen. 1975. Potassium activation in *Helix aspersa* neurons under voltage clamp: a component mediated by calcium influx. *J. Physiol.* (Lond.). 249:211–239.

Meves, H., and Y. Pichon. 1975. Effects of 4-amino-pyridine on potassium current in internally perfused giant axons of the squid. *J. Physiol.* (Lond.). 251:60P–62P.

Millecchia, R., and A. Mauro. 1969a. The ventral photoreceptor cells of *Limulus*. II. The basic photoresponse. *J. Gen. Physiol.* 54:310–330.

Millecchia, R., and A. Mauro. 1969b. The ventral photoreceptor cells of *Limulus*. III. A voltage clamp study. *J. Gen. Physiol.* 54:331–351.

Neher, E., and H. D. Lux. 1972. Differential action of TEA on two K-current components of a molluscan neurone. *Fleugers Arch. Eur. J. Physiol.* 356:87–100.

Pepose, J., and J. Lisman. 1976. Voltage-dependent potassium channels in *Limulus* ventral photoreceptors. Abstracts of the Association for Research in Vision and Ophthalmology. 3.

Schwartz, E. A. 1976. Electrical properties of the rod syncytium in the retina of the turtle. *J. Physiol.* (Lond.). 257:379–406.

Smith, T. G., W. K. Stell, and J. E. Brown. 1968. Conductance changes associated with receptor potentials in *Limulus* photoreceptors. *Science* (Wash. D. C.). 162:454–456.

Tsacopoulos, M., and J. Coles. 1977. Movement of potassium from photoreceptor to pigment cells during stimulation of the retina of the drone. Abstracts of the Association for Research in Vision and Ophthalmology. 120.

Werblin, F. S. 1975. Regenerative hyperpolarization in rods. *J. Physiol.* (Lond.). 244:53–81.

Yeh, J. Z., G. S. Oxford, C. H. Wu, and T. Narahashi. 1976. Interactions of aminopyridines with potassium channels of squid axon membranes. *Biophys. J.* 16:77–81.