Calcium-dependent Potassium Current in Barnacle Photoreceptor

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ABSTRACT When barnacle lateral eye photoreceptors are depolarized to membrane potentials of 0 to +50 mV in the dark, the plot of outward current through the cell membrane against time has two distinct maxima. The first maximum occurs 5-10 ms after the depolarization began. The current then decays to a minimum at ~500 ms after the onset of depolarization, and then increases to a second maximum 4-6 s after the depolarization began. If depolarization is maintained, the current again decays to reach a steady value ~1 min after depolarization began. The increase in current to the maximum at 4-6 s from the minimum at ~500 ms is termed the "late current." It is maximum for depolarizations to around +25 mV and is reduced in amplitude at more positive potentials. It is not observed when the membrane is depolarized to potentials more positive than +60 mV. The late current is inhibited by external cobaltous ion and external tetraethylammonium ion, and shows a requirement for external calcium ion. When the calcium-sequestering agent EGTA is injected, the late current is abolished. Illumination of a cell under voltage clamp reduces the amplitude of the late current recorded subsequently in the dark. On the basis of the voltage dependence and pharmacology of the late current, it is proposed that the current is a calcium-dependent potassium current.

INTRODUCTION

Injection of calcium ions into invertebrate photoreceptor cells produces inhibitory effects on the light-activated conductance that are comparable to the effects of light adaptation (Lisman and Brown, 1972; Bader et al., 1976). It has been proposed that an increase in intracellular calcium activity during illumination plays an important role in light adaptation.

A very different effect of raised intracellular calcium upon membrane ionic conductances is seen in central neurones of mollusks and mammals. Injection of calcium ions increases the potassium conductance of the cell membrane (Meech and Strumwasser, 1970; Krijević and Liesiewicz, 1972) and this action of raised intracellular calcium appears to be widespread in many other tissues, both excitable and inexcitable (Meech, 1976 and 1978). Injections of Ca\(^{++}\)/EGTA buffers into molluscan neurones indicate the threshold for
potassium channel activation to be in the region of $3 \times 10^{-7} - 9 \times 10^{-7}$ M Ca$^{++}$ (Meech, 1978). There is evidence that in barnacle photoreceptors the calcium entry during bright illumination is sufficient to activate an increase in membrane potassium conductance (Hanani and Shaw, 1977) and thus intracellular calcium appears to play a dual role in barnacle photoreceptor cells.

Meech and Standen (1975) have suggested that calcium-activated potassium channels in the cell membrane of Helix neurones act in concert with voltage-dependent calcium channels to form a calcium-dependent potassium conductance. On depolarization of these cells, voltage-dependent calcium channels in the cell membrane open. Calcium ions flow into the cell, and the rise in intracellular calcium activity acts to increase membrane potassium conductance. The outward current of potassium ions exceeds the inward calcium current, so that the net effect of depolarization is an outward current. The calcium-dependent potassium current shows a bell-shaped dependence on membrane potential, first increasing as the membrane is depolarized to more positive potentials and then decreasing as the electrochemical gradient for calcium is reduced. When the membrane is depolarized to potentials more positive than the calcium equilibrium potential, no calcium can enter the cell and therefore no outward calcium-dependent potassium current flows. Similar calcium-dependent potassium conductance mechanisms seem to operate in other molluscan neurones (e.g., Tritonia, Thompson, 1977) and in a sensory cell; the electroreceptor cells of the skate (Clusin et al., 1975).

Heyer and Lux (1976) have proposed an entirely different model of calcium-dependent potassium currents in molluscan neurones. They propose that the inward calcium current and the outward calcium-dependent potassium current are rigidly linked at the membrane itself. As calcium ions move inward through the membrane, they stimulate an outward movement of potassium ions. However, the model of Meech and Standen (1975), in which the activation of the potassium conductance is caused by a rise in intracellular calcium concentration rather than by the movement of calcium ions through the membrane, seems to fit recent evidence better (Meech, 1978; Eckert and Tillotson, 1978; Gorman and Thomas, 1980).

This study examines the possibility that a calcium-dependent potassium conductance forms a component of the membrane conductance of Balanus photoreceptors. Recordings from cells that are depolarized under voltage clamp in the dark show a slowly developing outward current that has many of the characteristics of a calcium-dependent potassium current. The relationship between membrane current and potential has a characteristic “N” shape that can be attributed to the combination of a calcium-dependent and a voltage-dependent potassium conductance (see Meech and Brown [1976]).

In Limulus ventral eye photoreceptors, prior illumination reduces the potassium conductance of the cell membrane (Lisman and Brown, 1971; Pepose and Lisman, 1978). A similar depression of potassium conductance by prior light is seen in barnacle, and seems to be a specific depression of the calcium-dependent potassium conductance.
MATERIALS AND METHODS

Lateral ocelli of the barnacle Balanus eburneus were dissected as described previously (Brown et al., 1970). Photoreceptor cells were penetrated with two micropipettes, each filled with 3 M potassium chloride; the membrane potential was measured differentially between one intracellular pipette and a third potassium chloride-filled micropipette placed close to the cell in the bath. The cells were voltage-clamped using a Philbrick 1022 high-voltage amplifier (Teledyne Philbrick, Dedham, Mass.) in a conventional circuit (see, for example, Brown and Cornwall [1975]). Given a square command pulse, the membrane potential would reach a new value in 1 ms; however, to eliminate instability on its rising and falling edges, the command pulse was slowed to a 1- or 10-ms rise time, depending on the time-course of the events being studied. The rise time of the current monitor was usually set at 1 ms, but was slowed to 10 or 25 ms when high frequency response was not required. Unless otherwise stated, depolarizing pulses were spaced at 1-min intervals.

Photoreceptor cells were constantly bathed with saline at a rate of 1-2 ml/min; bath volume was 2 ml. Normal saline had the composition 432 mM NaCl, 8 mM KCl, 20 mM CaCl₂, and 12 mM MgCl₂. Salines were equilibrated with gas of composition 97.5% O₂, 2.5% CO₂; NaHCO₃ was added to adjust the pH to 7.5. Cobalt salines were freshly prepared, with 12 mM CoCl₂ in place of MgCl₂ or with 30 mM CoCl₂ in place of 12 mM MgCl₂ and 12 mM NaCl. In the 50 mM tetraethylammonium (TEA) saline, TEA·Cl replaced 50 mM NaCl. In low Ca²⁺ saline, MgCl₂ replaced CaCl₂. Experiments were performed at room temperature (20-25°C).

The cells were illuminated via a fiber optic light guide using a quartz-iodine light source, neutral density filters, and a shutter system. Light intensities at the preparation were measured with a calibrated photodiode. Except where indicated, all experiments were performed in the dark on dark-adapted cells.

To study the effect of iontophoretic injection of the calcium-sequestering agent EGTA, cells were penetrated with one micropipette containing 500 mM EGTA with 10 mM Hepes at pH 7.8, and one micropipette containing 2 M KCl. Injection currents hyperpolarized the cells by ≤30 mV. During voltage clamp, the EGTA pipette recorded membrane potential, while the KCl pipette was used to pass the clamping current. For the EGTA iontophoresis experiments, saline buffered with Tris·Cl was used. This had composition 432 mM NaCl, 8 mM KCl, 20 mM CaCl₂, 12 mM MgCl₂, and 10 mM Tris·Cl at pH 7.8.

Where applicable, values are given as the mean ± twice the standard error.

RESULTS

Outward Current during Maintained Depolarization

When barnacle photoreceptor cells were depolarized in the dark to potentials of 0 to +50 mV, a transient outward current flowed through the cell membrane, reaching a maximum in 5–10 ms and then decaying to a relatively steady level (Fig. 2 a). If depolarization was maintained for several seconds, the current increased again, reaching a second maximum 4–6 s after pulse onset (Fig. 1 a).

In this paper, the late increase in outward current from the minimum at ~500 ms is referred to as the “late current,” and is measured as the difference between the current after 6 s of depolarization and the current at the minimum value. The late current was maximum for depolarization to around +25 mV;
when cells were depolarized to more positive membrane potentials, the late current was reduced. At potentials of +60 mV or above, no late current was observed (Fig. 1 a); the current showed an initial transient and then slowly decayed over the period of the depolarization with no secondary increase. In contrast to the behavior of the late current, the early transient outward current
increased monotonically with potential. The cell from which the records in
Fig. 1 were taken showed a particularly pronounced late current; for seven
cells that were depolarized for 6 s repetitively each minute to +15 mV, the
late current was 37 ± 13 nA. The measurement of the late current as the
difference between the current after 6 s of depolarization and the current at
the minimum value (after ~500 ms depolarization) is valid only if the
underlying membrane current does not decay significantly in this period. The
late current is absent at potentials of +60 mV and above, and here the
membrane current was seen to decay slightly in the period from 500 ms to
6 s. If such a decay also occurs at less positive potentials, the amplitude of the
late current will be slightly underestimated by this method of measurement.

Because the late current was only observed at potentials more negative than
+60 mV, the current-voltage relationship measured ≥1 s after the onset of
depolarization was markedly nonlinear. Fig. 1 b shows the current-voltage
(I-V) curves plotted from the traces in Fig. 1 a; the late increase in current at
potentials more negative than +60 mV is reflected in the pronounced N shape
of the I-V curve measured at 6 s. The slow decline of the current during
depolarizations to potentials more positive than +60 mV is reflected in the
crossover of early and late current curves at around this potential. Not all cells
had N-shaped I-V curves as dramatic as that of Fig. 1; of 11 cells in which an
I-V relationship was plotted, 7 showed a negative resistance region around
+20 to +60 mV; 4 had merely a prominent shoulder on the I-V plot at around
+25 mV.

The late current behaves as if it were a separate outward current component
with a bell-shaped I-V relation. This component develops late during pro-
longed depolarization and is summed with the slowly inactivating voltage-
dependent potassium current. The late current thus shows many of the
properties of the calcium-dependent potassium current of molluscan and other
cells (see Meech [1976 and 1978]).

**Effect of Cobaltous Ions**

In molluscan neurones, voltage-dependent and calcium-dependent potassium
currents can be distinguished on the basis of their susceptibility to agents,
such as cobaltous ion, that block calcium channels. Whereas the fast transient
outward current in barnacle photoreceptor was little affected by cobaltous

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**Figure 1 (opposite).** Clamping currents during long depolarizations. (a) Examples of current responses to voltage-clamp depolarizations to the potentials indicated (in mV) from the holding potential of −48 mV. The potential was not immediately returned to −48 mV but was held at −18 mV for 4 s after each depolarization as part of a tail-current experiment. Depolarizing pulses were spaced at 1-min intervals. The last trace shows the current response to a 30-mV hyperpolarization. Horizontal lines on each frame indicate zero current. Calibration: 200 nA, 2 s. (b) I-V curve of the photoreceptor from which the records in
(a) were obtained. Depolarizations of 6-s duration were spaced at 1-min intervals. Abscissa: membrane potential. Ordinate: membrane current, 500 ms (○),
2 s (□), and 6 s (●) after pulse onset.
ion application (Fig. 2 a-c), the late current was rapidly and reversibly suppressed. In the experiment illustrated in Fig. 2, 12 mM Co\(^{++}\) completely blocked the late current within 6 min. For six cells, cobalt saline (12 mM Co\(^{++}\) for three cells, 30 mM Co\(^{++}\) for three cells) reduced the late current to 11% ± 11% of the control value. Washing in normal saline restored the late
current to 64 ± 20% of the control value. With the removal of the late current, the I-V relation of the cells lost its N-shaped form (Fig. 3). This sensitivity to cobaltous ion is consistent with the late current being a calcium-dependent potassium current.

**Effect of Low-Calcium Saline**

If the late current passes through potassium channels activated by an influx of calcium ions, removal of extracellular calcium should reversibly abolish the current. When the cells were bathed in 0.2 mM calcium saline, the late current was abolished, but the effect took place only slowly. Fig. 4d shows the time-course of this effect in one cell. Return to normal 20 mM calcium saline led to a rapid recovery.

Fig. 4b shows the outward current during a 6-s depolarization to +15 mV in 0.2 mM calcium saline compared with the currents recorded in 20 mM calcium saline (Fig. 4a and c). There was no late current. The total current at the end of the 6-s depolarization was relatively large because of a reversible increase in leakage current in low-calcium saline.

A very slow but complete suppression of the late current was seen in all experiments with 0.2 mM calcium saline, and is consistent with the late current being a calcium-dependent potassium current.

**Iontophoretic Injection of EGTA**

If the late current is activated by a rise in intracellular calcium ion concentration, then injection of the calcium-sequestering agent EGTA should reduce or abolish the current. In all five cells studied, EGTA iontophoresis indeed caused a 98–100% reduction in late current amplitude (Fig. 5). EGTA iontophoresis also reduced the early transient outward current by 30–50%. In the experiment shown in Fig. 5, EGTA iontophoresis reduced the outward current at the end of a 6-s depolarization by more than would be expected if the only effect of the injection was the elimination of the late current. Such an
effect was observed in all the cells studied. In the experiment illustrated in Fig. 5, the outward currents after EGTA iontophoresis are smaller than the extrapolated "leakage" currents (Fig. 6). Thus, if the assumption of a linear leak is valid, the net current on depolarization is inward in an EGTA-injected cell. This phenomenon, a reduction of the outward current to levels below the extrapolated leak, implying a net inward current on depolarization, was observed in three out of the four cells in which the leak current was measured.

Outward Current to a Small Depolarization

Fig. 7a shows the membrane current that flowed when a cell was first hyperpolarized by 10 mV from the holding potential of −54 mV then depolarized by 10 mV. During the hyperpolarization a capacity transient was
Figure 4. Low calcium on the late current. (a) Current response (upper trace) to a 6-s depolarization to +15 mV from a holding potential of −45 mV. The lower trace shows the membrane potential. Calibration: 200 nA, 100 mV, 2 s. Normal saline. (b) As in (a), after 26 min in 0.2 mM Ca^{2+} saline. (c) As in (a), 15 min after return to normal saline. (d) Pulses were imposed at 1-min intervals and the late current (I_L) (measured as the difference between the current at 6 s and at its minimum) plotted as extracellular calcium was removed and restored.
followed by a steady inward ("leak") current. On depolarization, the initial current is equal and opposite to that flowing on hyperpolarization, but then the outward current increases to a steady level greater than the "leak."

The outward current to 10-mV depolarizations (with the leak, estimated with 10-mV hyperpolarizations, subtracted) was measured as cobaltous ions and then TEA ions were applied. Both treatments blocked the outward current completely, leaving only the leak (Fig. 7 b). This result suggests that the current produced by a depolarization of 10 mV from the resting potential is a pure calcium-dependent potassium current, with no contribution from the Co$^{2+}$-resistant current.

Extracellular TEA is known to block calcium-dependent potassium currents in Helix and Aplysia neurones (Meech and Standen, 1975; Hermann and Gorman, 1979), although in some other cells the calcium-dependent potassium current is very resistant to TEA (Thompson, 1977; Aldrich et al., 1979).

\begin{figure}
\centering
\includegraphics{figure5}
\caption{Effect of EGTA injection on the late current. Current responses to voltage-clamp depolarizations to the potentials indicated (in mV) from the holding potential of -33 mV. The first trace in each column shows the current response to a 10-mV hyperpolarization. Depolarizations were spaced at 1-min intervals. Calibration: 100 nA, 10 s. (a) Before iontophotic injection of EGTA. (b) After injection of EGTA (9 x 10^{-4} C).}
\end{figure}
Inactivation and Recovery of the Late Current

When cells were depolarized for ≥1 min, the outward current slowly decayed to a final steady value (Fig. 8 b). A 6-s test depolarization a few seconds after this inactivating depolarization produced a normal voltage-dependent current but no late current (Fig. 8 c). If test depolarizations were given every minute, the late current was seen to recover over the course of 5–15 min (Fig. 8 e).

The 6-s depolarizations used in this study caused appreciable inactivation of the late current. For this reason, depolarizations of >10 mV amplitude were always separated by periods of ≥1 min at the holding potential. In one experiment, the membrane potential of a cell was held at the holding potential of −40 mV for 5 min to allow recovery from any previous inactivation and then shifted to +20 mV twice, in a pair of 6-s depolarizations separated by a period of 24 s at the holding potential. The late current during the second depolarization was 24% smaller than the late current during the first depolarization. When the same depolarizations were separated by 54 s at the holding potential, the current traces were, however, indistinguishable.

Action of Light on the Membrane Potassium Conductance

Prior illumination reduces the potassium conductance of Limulus ventral photoreceptor cell membrane (Lisman and Brown, 1971; Pepose and Lisman, 1978). Fig. 9 illustrates an experiment to investigate the effects of prior illumination upon both components of potassium conductance in barnacle
photoreceptor. The late current was measured as the outward current during a 10-mV depolarization from the holding potential of $-52$ mV, corrected for leakage current, whereas the amplitude of the early transient current during depolarization to $+8$ mV gave a measure of the cobalt-resistant current. At

![Diagram](image)

**Figure 7.** Outward current during a small depolarization. (a) Current response (upper trace) to a 10-mV hyperpolarization, followed by a 10-mV depolarization, from a holding potential of $-54$ mV. Calibration: 20 nA, 1 s. Lower trace: membrane potential, calibration: 100 mV. Control response in normal saline. (b) Outward current, measured as the difference between the currents elicited by 10-mV depolarizations and hyperpolarizations, as in (a), plotted to show the time-course of the effect of application of first Co$^{2+}$ (30 mM) and then TEA (50 mM).

Each of the three vertical arrows, the cell was illuminated with an adapting light of 15-s duration, the illumination terminating 5 s before the next current measurement. Illumination produced a clear reduction in the late current but had no significant effect upon the early transient.
Figure 8. Inactivation and recovery of the late current. (a) Current response to a depolarization to +15 mV from a holding potential of -51 mV. Calibration for a, c, and d: 100 nA, 1 s. (b) Current response (upper trace) to a maintained depolarization to +15 mV. Calibration: 40 nA, 20 s. Lower trace: membrane potential. Calibration: 50 mV. (c) Current response to a depolarization to +15 mV. This depolarization was imposed 5 s after the end of the 2-min depolarization illustrated in (b). (d) Current response to a depolarization to +15 mV imposed 17 min after the 2-min depolarization shown in (b). (e) Plot of the late current $I_L$ (measured as the difference between the current at 6 s and at its minimum) before and after the 2-min depolarization. Each point is taken from a single 6-s depolarization to +15 mV from the holding potential of -51 mV.

The degree of reduction of the late current was graded with the intensity of illumination. A xenon flash delivered 5 s before each current reading (intensity at the preparation, $1.6 \times 10^{-3}$ J/cm² per flash) reduced the late current by 3% in one cell, whereas in a different cell, a 35-s illumination with steady quartz-halogen light ending 10 s before each current reading (total energy in the
35-s illumination, $1.6 \, \text{J/cm}^2$) reduced the late current by 92%. Illumination reduced the inward current during a 10-mV hyperpolarization. In the experiment of Fig. 9, the inward current during a 10-mV hyperpolarization was reduced by 10% immediately after the first flash (which reduced the late current by 34%) and by 15% after the third flash (which reduced the late current by 80%).

**Figure 9.** The effect of light adaptation on dark currents. *Late current:* the late current measured as the outward current during a 10-mV depolarization from the holding potential of $-52 \, \text{mV}$, minus the inward (leak) current during a 10-mV hyperpolarization. *Early transient:* the peak (at $\sim 10 \, \text{ms}$ after pulse onset) of the early outward current transient evoked by a 40-ms depolarization to $+8 \, \text{mV}$. Pulses in each case were delivered at 30-s intervals. The adapting light, delivered at each *arrow,* lasted 15 s and ended 5 s before the next pulse. A quartz-iodine light source, intensity at the preparation $4.6 \times 10^{-2} \, \text{W/cm}^2$, was passed through a blue filter (Wratten 47B; Eastman Kodak, Rochester, N. Y.) to ensure rapid return of the photocurrent to its resting level at light-off. During illumination the cell was clamped at the holding potential of $-52 \, \text{mV}$; the numbers by each *arrow* give the plateau photocurrent evoked by the adapting light in nA, and indicate that the sensitivity of the cell was increasing during the course of the experiment.

**DISCUSSION**

The results presented in this paper are evidence for a calcium-dependent potassium current in barnacle photoreceptor cells. Such a current requires the presence in the cell membrane of both voltage-dependent calcium channels and calcium-activated potassium channels. Hanani and Shaw (1977) have found that the rise in intracellular calcium concentration that occurs in barnacle photoreceptor cells in the light can activate a potassium conductance directly. Thus, there appear to be two mechanisms that allow an influx of calcium ions into barnacle photoreceptor cells. Upon illumination, light-activated channels allow both sodium and calcium ions to pass (Brown et al.,
1970; Brown and Blinks, 1974). In addition, upon depolarization in the dark, calcium can enter through voltage-dependent channels. Either flux can raise intracellular calcium to levels high enough to activate the potassium conductance.

The time-courses of the two calcium entries are, however, very different. The increase in intracellular calcium concentration on illumination is rapid. The peak of intracellular calcium concentration occurs \( \sim 500 \) ms after the onset of illumination (Brown and Blinks, 1974). This rapid increase in intracellular calcium concentration evokes a rapid potassium current through the calcium-activated potassium channels (Hanani and Shaw, 1977). The calcium-dependent potassium current reported here is, on the other hand, extremely slow, reflecting an increase over 4–6 s of the intracellular calcium concentration at the membrane in which the potassium channels are located. This slow increase of \( \text{Ca}^{2+} \) at the site of action may simply reflect the small amplitude of the voltage-dependent calcium current in these cells. It may, however, reflect the time taken by calcium ions to diffuse from a remote site of entry to the region of the cell membrane where the potassium channels are located. A diffusion distance of \( 15 \mu \text{m} \) through cytoplasm is enough to produce a delay of 5–10 s in potassium current activation in an \textit{Aplysia} neurone (Andresen et al., 1979).

The calcium-dependent potassium current reported here shows a bell-shaped dependence on membrane potential, first increasing and then declining to disappear at a potential of about \( +60 \) mV (Figs. 1 and 3). Such a bell-shaped relationship is common to many processes that depend on calcium entry into cells, for instance the aequorin light response in squid axon. The "null potential" above which the calcium entry is not observed is often taken to be the calcium equilibrium potential; however, in many instances the intracellular calcium concentration corresponding to this potential is surprisingly high (Baker et al., 1971). \( +60 \) mV, the null potential of the calcium-dependent potassium current in barnacle photoreceptors, corresponds to an intracellular calcium concentration of 0.2 mM, an extremely high value. It might be that the calcium concentration close to the membrane does indeed rise to this level during the depolarization. Calcium concentrations of this magnitude are not unknown in invertebrate photoreceptors: light can cause the concentration of calcium inside \textit{Limulus} ventral photoreceptors to rise to between 0.1 and 0.7 mM (Brown et al., 1977). Alternatively, the null potential may not represent the calcium equilibrium potential. The calcium conductance of the cell membrane may be reduced at potentials more positive than +25 mV and may become extremely small above +60 mV. Such a mechanism has been suggested for the calcium conductance of squid axon membrane (Baker et al., 1971). Hagiwara and Byerly (1981) have shown that when the concentration gradient of an ion across the cell membrane is as extreme as is the case for calcium, the constant field model of Hodgkin and Katz (1949) predicts just this behavior: a progressive fall in conductance as the membrane potential is shifted to more positive potentials, with the ionic current falling to a small fraction of its maximum amplitude at a membrane potential that is still 50–100 mV more negative than the equilibrium potential.
Iontophoretic injection of the calcium-sequestering agent EGTA eliminates the late current of barnacle photoreceptors, as is expected if the current is a calcium-dependent potassium current. However, the reduction in outward current after EGTA injection is greater than that expected if the only effect of EGTA injection is the abolition of the late current. Indeed, in three out of four cells, the outward current after EGTA injection was smaller than the extrapolated leakage current, implying a net inward current on depolarization. The greater than expected reduction in the outward current may be due to a combination of three factors. (a) The late current, estimated as the increase in outward current from its minimum value, may be an underestimate of the calcium-dependent potassium current. (b) EGTA injection may reduce the amplitude of the maintained voltage-dependent outward current. The early transient voltage-dependent outward current is reduced 30-50% by EGTA injection. (c) In molluscan neurones and Paramecium, the inward current through the calcium conductance is increased in amplitude and duration when intracellular calcium accumulation is prevented (Tillotson, 1979; Adams and Gage, 1980; Brehm et al., 1980). The injection of EGTA into barnacle photoreceptor cells may increase the amplitude and duration of the calcium current, thus reducing the net outward current.

**Possible Artefacts Due to Intercellular Junctions**

The lateral eye of Balanus eburneus contains three photoreceptor cells that are electrically coupled. The coupling is a possible source of artefacts in voltage-clamp studies, because the current recorded will differ from the current flowing through the membrane of the clamped cell because of current flowing to the other two cells. If it is assumed that the I-V relations of the three cells are identical, and that the intercellular conductances are constant, it is possible to calculate the I-V relation of the membrane of one cell from the measured data (Brown et al., 1971). Performing this calculation on the N-shaped I-V relation of barnacle photoreceptors, using the highest value of intercellular conductance found by Brown et al., produces quantitative but not qualitative differences from the measured I-V curve.

A more serious problem would arise if the intercellular conductances were time- and voltage-dependent. If depolarization of one cell slowly increased the value of the intercellular conductances, the increased current flowing to the other two cells would appear as a late current. However, it is difficult to see why such a mechanism should operate only at membrane potentials more negative than +60 mV, be blocked by cobaltous ion or EGTA injection, and have a requirement for external calcium, unless the intercellular conductance increase itself were triggered by an entry of calcium ion into the cytoplasm. In fact, intercellular low resistance junctions in various tissues are known to fall in conductance when calcium enters the cytoplasm, either because of a direct action of calcium or by a resultant cytoplasmic acidification (Rose and Loewenstein, 1975; Turin and Warner, 1977). It therefore appears unlikely that the late current is an artefact caused by intercellular conductances.
Can Intracellular Calcium Reduce $G_K$?

In molluscan neurones, a rise in intracellular calcium concentration can in some circumstances reduce the potassium conductance ($G_K$) of the membrane. This effect is slow, so that an injection of calcium ions may first increase, and then reduce, the membrane potassium conductance. (Heyer and Lux, 1976; Gorman and Thomas, 1980). An increase of intracellular calcium is known to reduce the calcium conductance of molluscan neurones (Akaike et al., 1978), and this reduction in calcium conductance may be responsible for the inhibition of the calcium-dependent potassium current by raised intracellular calcium. However, calcium injection seems to be equally effective at reducing the voltage-dependent potassium current (Gorman and Thomas, 1980). Meech (1978) has suggested that the inhibition of potassium currents by calcium injections is not a direct effect of calcium but is a result of the fall in intracellular pH that occurs after calcium injection (Meech and Thomas, 1980).

A slow inhibitory effect of raised intracellular calcium on the calcium-dependent potassium current may be responsible for two of the results presented in this paper: the inactivation of the calcium-dependent potassium current during prolonged depolarization, and the inhibition of the calcium-dependent potassium current by light. The intracellular calcium concentration of these cells rises in the light (Brown and Blinks, 1974), and this increase can activate a potassium conductance (Hanani and Shaw, 1977). It may be that just as large calcium injections first increase and then depress the potassium conductance of Aplysia neurones (Gorman and Thomas, 1980), light first increases, then depresses, the potassium conductance of barnacle photoreceptors.

The inactivation of the calcium-dependent potassium current during prolonged depolarization may be the result of a slow, secondary effect of the increase in intracellular calcium during the depolarization. If both inactivation and the inhibition of the calcium-dependent potassium current by light are the result of raised intracellular calcium, then the calcium-dependent potassium current should recover from both light and inactivation at about the same rate. A comparison of Figs. 8 and 9 shows this to be true, although a second, slow component in the recovery from inactivation that is apparent in Fig. 8 is absent in the recovery of the calcium-dependent potassium current from illumination.

The potassium current flowing through the membrane of Limulus ventral photoreceptor cells in the dark shows an N-shaped dependence on membrane potential. The current is reduced by prior light (Lisman and Brown, 1971). An N-shaped dependence of potassium current on membrane potential does not necessarily indicate that a calcium-dependent potassium current contributes to the total current (Aldrich et al., 1979). Nevertheless, it may be that a calcium-dependent potassium current contributes to the outward current in Limulus photoreceptors as it does in Balanus. Pepose and Lisman (1978) have reported that iontophoretic injection of calcium into Limulus photoreceptors...
reduces the currents recorded on depolarization, in much the same way as does prior illumination. They regard this result as strong evidence against the presence of a calcium-dependent potassium current. However, as noted above, large injections of calcium may reduce all components of potassium conductance (Gorman and Thomas, 1980). Meech and Brown (1976) have suggested that a long, slow iontophoretic injection of calcium may have a greater effect on intracellular pH than it does on the concentration of free calcium. If this is the case, then the effect of calcium iontophoresis would be to depress all potassium currents (Meech, 1978).

Function of the Calcium-dependent Potassium Current in Barnacle Photoreceptors: a Speculative Model

The light response of a barnacle photoreceptor consists of a transient depolarization of up to 500 ms duration, followed by a plateau response (Brown et al., 1970). Since the calcium-dependent potassium current only appears after 500 ms of depolarization, its role must lie in modulating the plateau response.

One component of the calcium-dependent potassium conductance system does, however, appear to play a role in the transient photoreponse. The large rise in intracellular calcium that occurs in the light (Brown and Blinks, 1974) can increase the potassium conductance of the membrane and thus accelerate the falling phase of the transient depolarization (Hanani and Shaw, 1977). One role of the calcium-activated potassium channels, therefore, may be to reduce the duration of the transient response to bright lights. However, this role does not require the presence in the membrane of voltage-dependent calcium channels.

To find the role of the calcium-dependent potassium current in modulating the plateau response, it is helpful to consider what the behavior of the cell would be if the only conductances present in the cell membrane were the light-induced conductance and a linear leak. In this case, the depolarization produced by illumination would be directly proportional to the light-induced current. In the presence of an active potassium current, however, successive increments of light-induced current produce steadily less depolarization, because the membrane potassium conductance increases steadily with depolarization (Figs. 1 and 3). The cobalt-resistant current, however, does not activate until the membrane is depolarized to around -25 mV (Fig. 3). For small depolarizations from the resting potential, only the calcium-dependent potassium current is activated (Fig. 7). The function of the calcium-dependent potassium current may therefore be to ensure a smooth and graded increase in membrane potassium conductance with membrane potential over the whole of the working range of the plateau response to light, -60 mV to 0 mV (Brown et al., 1970).

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REFERENCES


