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A POTASSIUM CONTRIBUTION TO THE RESPONSE OF THE BARNACLE PHOTORECEPTOR

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SUMMARY

1. Intracellular recording from photoreceptors in the lateral eye of the barnacle show a brief negative-going 'dip' shortly after the onset of the late receptor potential. This phase can sometimes result in a hyperpolarization relative to the resting membrane potential.

2. The dip is prominent in light-adapted cells and is reduced by dark-adaptation. Low extracellular Ca^{2+} also reduces it.

3. The amplitude of the dip changes inversely with the K^+ concentration in the saline.

4. The amplitude of the dip depends on the membrane potential, with a reversal potential near -80 mV.

5. K^+ blocking agents such as quinine and quinidine reduce or abolish the dip.

6. These observations indicate that the dip is due to a brief increase in K^+ conductance which may be dependent on an influx of Ca ions. The fast decay of this phase may be brought about by a rapid uptake of Ca^{2+} by an intracellular mechanism.

INTRODUCTION

Invertebrate photoreceptors respond to light by an increase in membrane conductance. In most cases (e.g. in *Limulus* and barnacle) the photoreceptor depolarizes on illumination, due to an increase in membrane conductance, predominantly to Na ions (Millecchia & Mauro, 1969; Brown, Hagiwara, Koike & Meech, 1970). In some invertebrate eyes (e.g. scallop), however, there are photoreceptors which respond to light with a hyperpolarization, possibly the result of a rise in the intracellular Ca ion concentration which leads to an increase in K permeability (P_K)

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(Gorman & McReynolds, 1974; McReynolds & Gorman, 1974). Recent studies have shown that depolarizing photoreceptors may also have permeability changes to ions other than sodium, particularly to potassium. Brown & Mote (1974) found that light induces a change in membrane permeability to ion(s) other than Na in the *Limulus* ventral eye, and they suggest that light may increase P_K. Recently Detwiler (1976) showed that *Hermissenda* photoreceptors have two distinct phases in their responses, which involve changes in P_K.

Measurements of ion fluxes indicate that light can increase the rate of potassium movement across the photoreceptor membrane. Holt & Brown (1972) found that light enhances K^+ efflux from the *Limulus* ventral eye, and they postulated the existence of a transient increase in P_K during or shortly after illumination. Similar results were obtained by Stieve, Malinowska & Sonemann (1974) for the crayfish retina.

If, in fact, P_K increases on illumination it could have an effect on the receptor potential, since such a change would tend to result in hyperpolarization. In the present paper we attempt to show that there is a relatively brief phase of increased P_K which is induced by a prior calcium influx. This phase modifies the wave form of the response and may have a functional significance.

METHODS

Details of the preparation have been dealt with at length in a previous paper (Hanani & Hillman, 1976). The lateral ocelli of *Balanus amphitrite* were excised together with a short section of nerve. The ocelli were placed in a chamber perfused with either sea water or barnacle Ringer (Brown *et al.* 1970) with various modifications. Normal Ringer contained 10 or 20 mM-Ca; low-Ca Ringer contained 0·5–2 mM-Ca. In several experiments the concentration of K was also varied between 0·1 and 32 mM. Other substances were also added to the perfusate and include quinine, quinidine and tetraethylammonium-chloride (TEA). Before the experiments were begun the preparation was bathed for about 3 min with 1·5% collagenase and 1·5% protease in sea water. The cornea was then removed and a corneal approach was used for intracellular recording with micropipettes containing either 2 M-KCl or 4 M-K-acetate. In a few experiments electrodes filled with CsCl (2M) or TEA-Cl (1–1·5 M) were used for recording and for intracellular iontophoretic injection. In experiments designed to measure reversal potential for different phases of the response, two electrodes were glued together (tip separation less than 20 μm) and inserted into a single photoreceptor. One electrode was used to pass current to change membrane potential to different levels, while the second electrode served to record potential changes. The light stimulus was presented during the plateau of the step change in membrane potential. A quartz-iodine lamp provided white light whose unattenuated intensity was about 1×10^{16} quanta cm^{-2} sec^{-1} nm^{-1} at 550 nm at the photoreceptor. Intensities are given as the logarithms of their ratios to this value.

RESULTS

When a very bright light is presented to a previously light-adapted barnacle photoreceptor, a negative-going 'dip' appears on the depolarizing response about 100–200 msec after the onset of the stimulus (Fig. 1*A*). This phase lasts approximately 200 msec and is unlikely to be the result of an electrogenic sodium pump, which has a time course of the order of seconds to minutes (Koike, Brown & Hagiwara, 1971). In some cases this

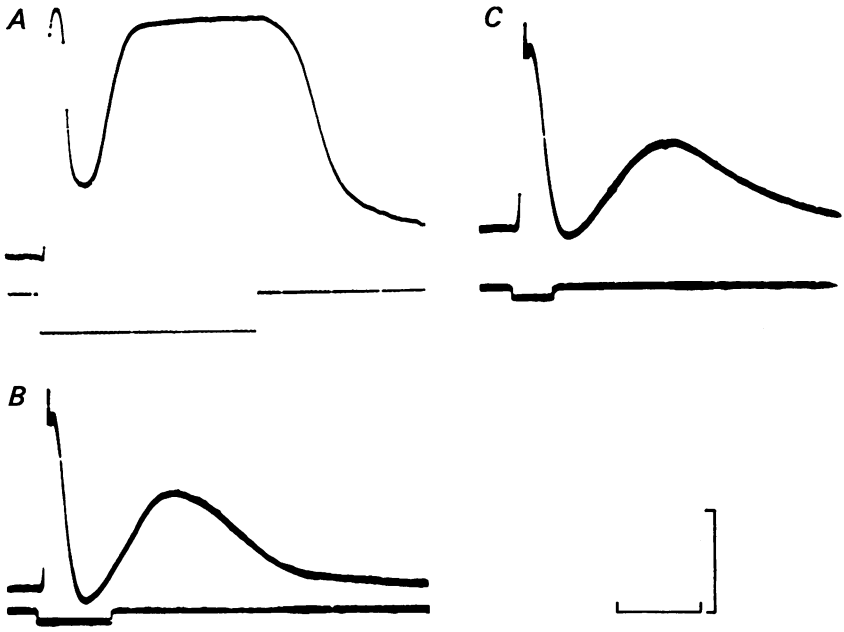

Fig. 1. Examples of the dip from two different cells. Both cells were light-adapted. *A*, negative-going dip; flash intensity log $I = -1{\cdot}0$, flash duration 850 msec. *B*, hyperpolarizing dip; log $I = 0$, duration 75 msec. *C*, hyperpolarizing dip; log $I = 0$, duration 40 msec. Same cell as in *B*. Note that the dip occurs approximately at the same time after the onset of the flash, irrespective of the flash duration. Calibration, vertical bar 20 mV for *A–C*; horizontal bar, 400 msec for *A*, 100 msec for *B* and *C*.

negative-going potential is sufficient to result in a net hyperpolarization of the membrane (Fig. 1 *B*). This hyperpolarizing dip can also be observed when short (10–50 msec) flashes are used, as shown in Fig. 1*C*. In the following sections results will be presented which demonstrate the effects of various experimental parameters on the dip.

The effect of light- and dark-adaptation

The negative-going dip depends strongly on the state of adaptation of the cell. In the dark-adapted state the dip is small or absent but it becomes more conspicuous as the cell is light-adapted. In Fig. 2*A* the uppermost recording is the response to a short flash which was given to a cell which was dark-adapted for 8 min. The flash was repeated every 5 sec and the superimposed recordings show that the response becomes narrower and the dip more pronounced as light-adaptation progresses.

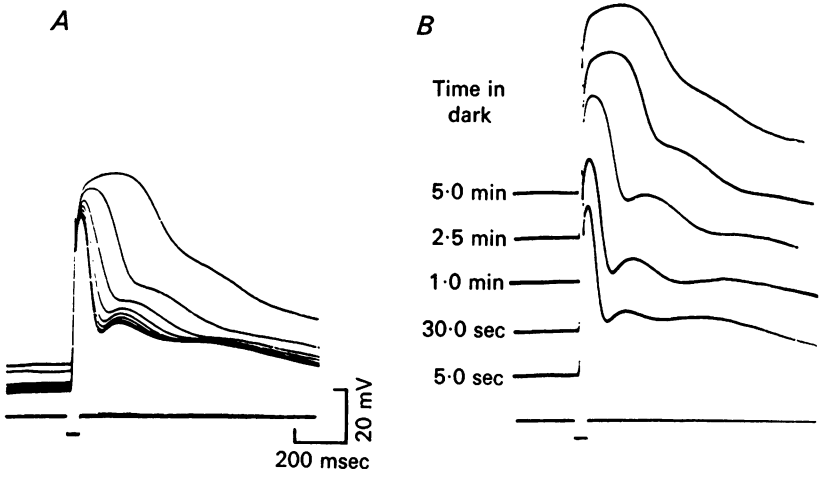

Fig. 2. Light- and dark-adaptation. *A*, development of the dip in a dark-adapted cell (8 min in the dark). A flash ($\log I = -1{\cdot}0$, 40 msec duration) was presented every 5 sec. The dip increases and the response narrows with light-adaptation. There is also a reduction in the depolarizing response.

B, disappearance of the dip with dark-adaptation. Same cell and conditions as in *A*. Before each measurement the cell was light-adapted to produce saturation of the dip. The recordings were taken after the time in the dark indicated by the numbers to the left of each recording. The responses are displaced on the vertical axis for clarity of illustration.

The effects of light adaptation are reversed in the dark with a time course of several minutes. The recovery of the response in the dark, for the same cell and conditions as Fig. 2*A* is shown in Fig. 2*B*.

External Ca concentration ($[Ca^{2+}]_o$)

An intense conditioning light usually reduces photoreceptor sensitivity and recent results have shown that this reduction can be significantly inhibited by low $[Ca^{2+}]_o$ (Hanani & Hillman, 1976). The present experiments are an attempt to find out if low $[Ca^{2+}]_o$ could also inhibit the effects of light on the response noted in the previous section. Lowering $[Ca^{2+}]_o$

from 20 to 1 mM abolished the dip (Fig. 3) and the effect was completely reversible (not shown). This result suggests that Ca^{2+} influx may play a role in producing the dip. It is also evident that low $[Ca^{2+}]_o$ and dark-adaptation act similarly (cf. Fig. 2*B*).

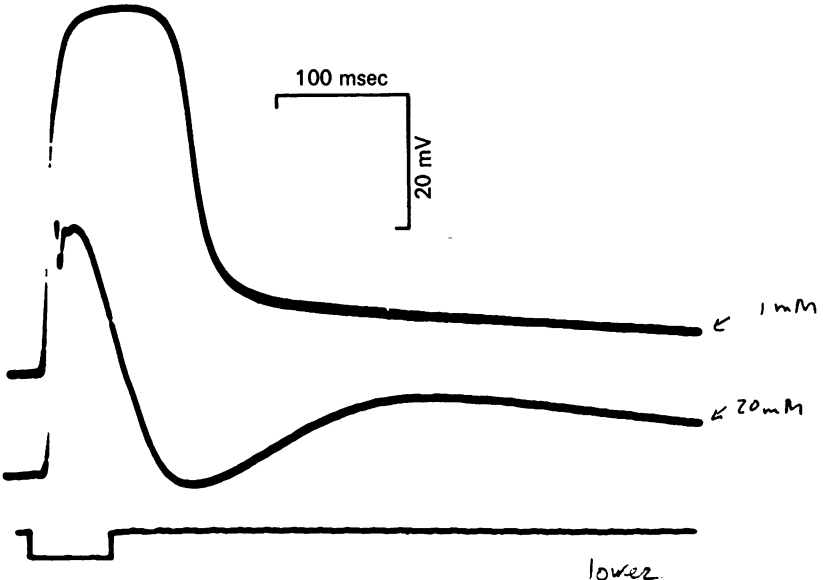

Fig. 3. The effect of changes in $[Ca^{2+}]_o$ on the dip. In the ~~upper~~ recording $[Ca^{2+}]_o$ = 20 mM, in the ~~lower~~ upper recording $[Ca^{2+}]_o$ = 1 mM for the same cell. Log I = −1·5, duration 60 msec. Lowering $[Ca^{2+}]_o$ abolishes the dip. The result was reversible. The responses were displaced on the vertical axis for the clarity of illustration.

External K concentration ($[K^+]_o$)

The previous results indicate that the dip may be due to a transient increase in membrane permeability to a cation with an equilibrium potential more negative than the resting level and/or to a decrease in the permeability to a cation with an equilibrium potential more positive than the resting potential. Anions have not been considered since Brown *et al.* (1970) have shown that replacement of chloride, the only anion present in the medium, by an impermeant anion did not affect the photoreceptor's current–voltage curve appreciably. The most likely relevant cation for the dip response is potassium, whose equilibrium potential is more negative than resting level. The effect of changes in $[K^+]_o$ are presented in Fig. 4. Normal $[K^+]_o$ is 8 mM, at lower $[K^+]_o$ values the dip becomes more negative and the response narrower. At higher than normal $[K^+]_o$ values the dip disappeared. Thus, the smaller the K^+ driving force, the smaller the dip, and vice versa.

Brown & Ottoson (1976) have found that the light response of the barnacle photoreceptor was abolished in the absence of K^+ in the saline. Our experiments, however, do not show a considerable change in the amplitude of the response in low $[K^+]_o$ (Fig. 4). This apparent disagreement can be attributed to the following differences in experimental conditions: (1) K^+-free salines were not used in the present experiments, $[K^+]_o$ was lowered only to 0·1 mM; (2) low $[K^+]_o$ salines were used for periods of up to 15–20 min, after which the response reached steady state, while the effects described by Brown & Ottoson become apparent only after 20–30 min in

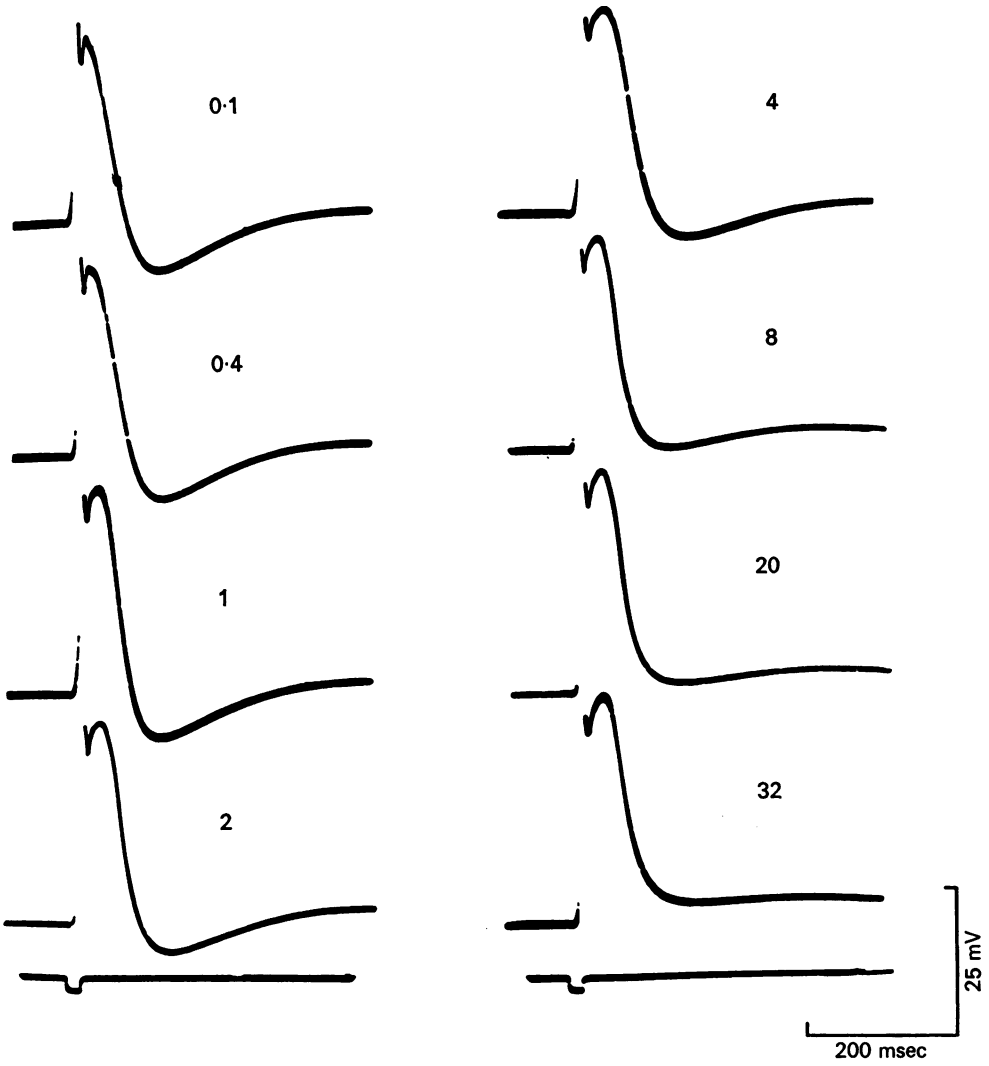

Fig. 4. Effect of changing $[K^+]_o$ on the dip. Concentrations of K^+ in the saline in mM are indicated above each recording. Log $I = -0·8$, duration 20 msec. Increasing $[K^+]_o$ abolishes the dip, lowering $[K^+]_o$ enhances it. These effects were reversible.

K^+-free saline; (3) in Brown & Ottoson's experiments the preparation was constantly light-adapted, whereas in the present work the preparation was kept in the dark during most of the time and was light-adapted only before the presentation of the test flash.

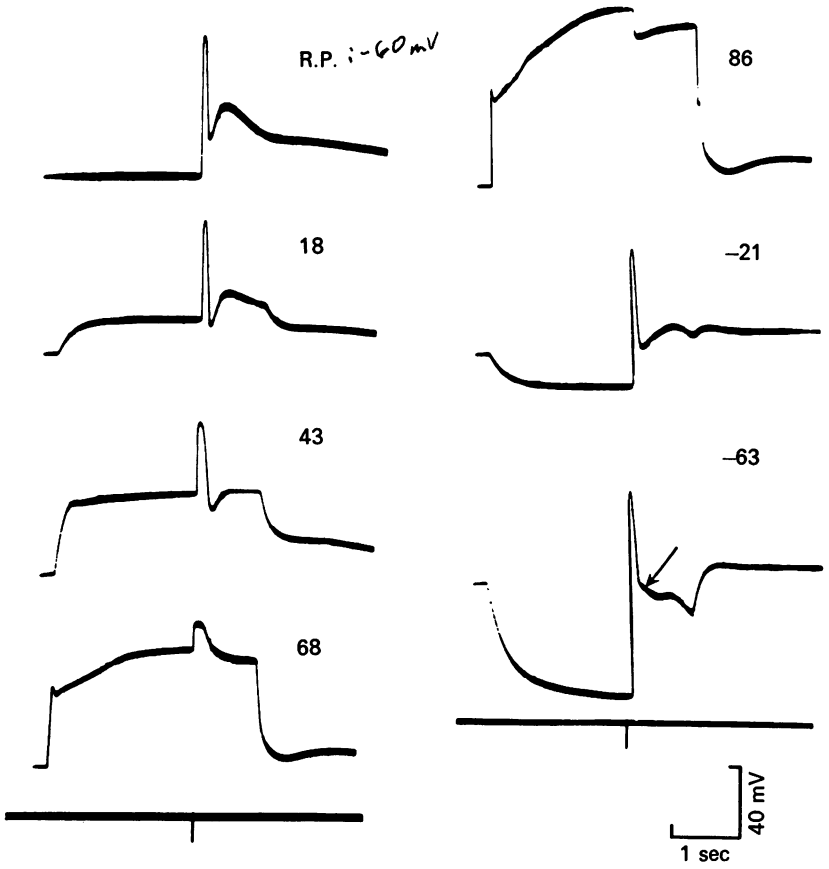

Fig. 5. The effect of intracellularly injected current on the response. Current pulses of 3 sec duration and different magnitude and polarity were passed through one electrode and the changes in membrane potential were recorded with a second electrode. The light stimulus ($\log I = 0$, duration 40 msec) was presented during the plateau phase of the membrane potential to each current. The membrane potential at the plateau, relative to the resting level (R.P.) is indicated above each recording, the values are in mV. Resting potential of this cell is -60 mV. The arrow points to the reversal of the dip, when the membrane potential was shifted to -63 mV below the resting level.

Membrane potential

Fig. 5 shows that as the membrane is depolarized by external current, the dip initially becomes more pronounced, consistent with a reversal potential more negative than the resting level. The late phase of the

response (the 'plateau') reverses at about −17 mV and the whole response is reversed at about +20 mV (cf. Brown *et al* 1970). Above −20 mV (40 mV depolarization) the dip size starts to diminish until it disappears around 0 mV. At hyperpolarizing potentials the response amplitude increases whereas the dip diminishes until it disappears at about −80 mV. Below this level a positive-going phase seems to be present (arrow), indicating the reversal of the dip. Thus, while the positive-going phases of the response disappear at only one potential, the dip is abolished at two membrane potentials, one above the resting level, and one below it. The dependence of the dip on membrane potential more negative than the resting level is thus consistent with its being due to K; the behaviour above −20 mV will be considered in the Discussion.

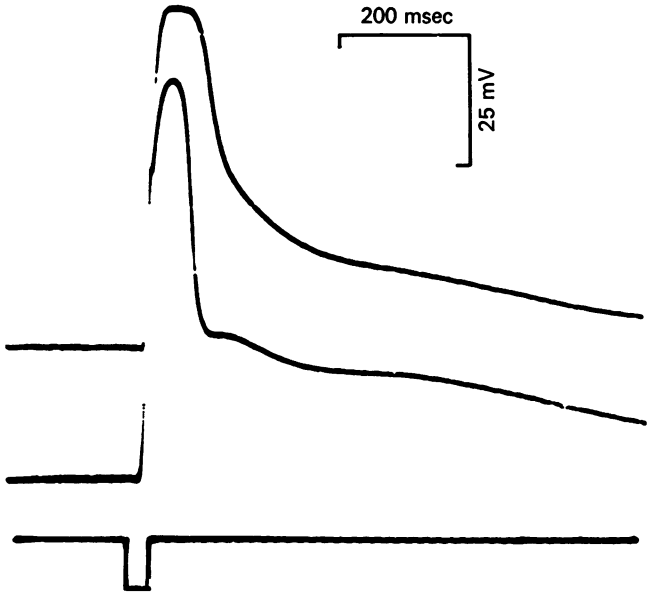

Fig. 6. Intracellular injection of cesium ions. The recording electrode was filled with 2 M-CsCl. The responses are to flashes before (lower recording) and after injection of 30 nA for 5 min, $\log I = -1{\cdot}0$, duration 40 msec. Note the broadening of the response. This effect was not reversible. The responses were displaced on the vertical axis for the clarity of illustration.

K^+-blocking agents

Several substances are known to block potassium conductance. Tetraethylammonium (TEA) is an ion which blocks the slow K^+ channels in various nerve membranes (Hille, 1970). In the barnacle photoreceptor the addition of TEA at concentrations of up to 20 mM did not have a measureable effect on the response. On the other hand, intracellular iontophoretic

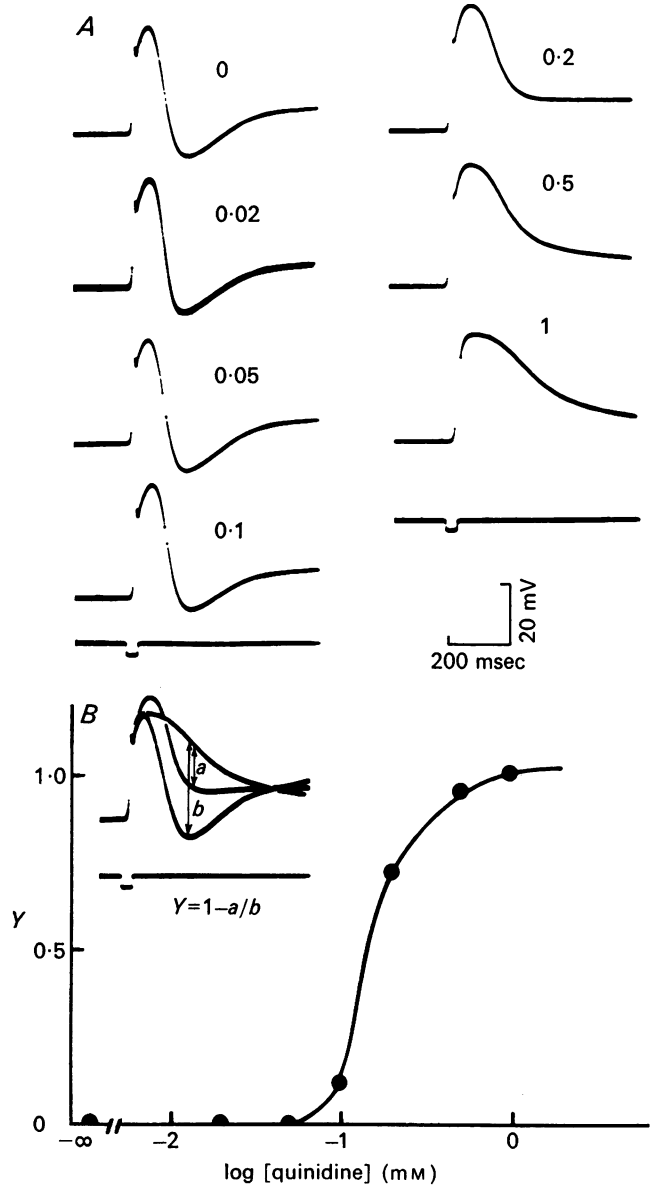

Fig. 7. The effect of K^+-blocking agent quinidine, on the response. Since the effect is not reversible, the concentrations were increased gradually and the preparation was kept at each concentration until the shape of the response reached a steady level (within 10–20 min). *A*, the numbers above each recording give the concentration of quinidine in mM; $\log I = -0{\cdot}5$, flash duration 30 msec. *B*, dose–response curve for the effect of quinidine on the response using the results of *A*. The definition of Y is shown in the insert. Similar curves were obtained for quinine.

application of TEA, through the recording electrode (5 nA for about 20 min), caused a broadening of the response and diminished the size of the dip. A similar effect was observed after the injection of cesium ions, which are also known to block K^+ channels (Chandler & Meves, 1965; Dubois & Bergman, 1975), as shown in Fig. 6.

A relatively large effect on the form of the response was observed when quinine or its isomer quinidine were added to the external medium. These substances are known to suppress the calcium-induced increase in P_K in red blood cells (Amando-Hardy, Ellory, Ferreira, Fleminger & Lew, 1975). In the barnacle photoreceptor their effect on the response resembles that of low $[Ca^{2+}]_o$ or elevated $[K^+]_o$ (Fig. 7*A*). In contrast to the effects of $[Ca^{2+}]_o$ and $[K^+]_o$ the effects of quinine and quinidine were not reversible. Dose–response curves for quinine and quinidine were determined by gradually increasing the drug concentration (Fig. 7*B*). The curves for both substances are very similar and show half saturation value at 0·15–0·2 mM, which is the same range obtained for red blood cell (V. L. Lew, personal communication).

DISCUSSION

In this paper we have presented evidence consistent with the interpretation that in the barnacle photoreceptor, light induces an outward K^+ current resulting from increased intracellular Ca^{2+} concentration. Detwiler (1976) observed a hyperpolarizing phase in the *Hermissenda* photoreceptor, which he also attributed to increased P_K. As in the case of *Hermissenda*, the negative-going potential in the barnacle photoreceptor is enhanced by light-adaptation (Fig. 1), varies inversely with $[K^+]_o$ (Fig. 4) and is reversed by externally applied current at a potential more negative than the resting potential (Fig. 5). In addition, we show that small depolarizing currents increase the amplitude of the dip (Fig. 5) and that K^+-blocking agents reduce or abolish it (Figs. 6, 7).

A Ca^{2+}-induced K^+ current is known to exist in various types of neurones (Meech, 1972, 1974; Krnjević & Lisiewicz, 1972; Krnjević, Puil & Werman, 1975; Brown & Brown, 1973) and was suggested for the scallop photoreceptor (Gorman & McReynolds, 1974; McReynolds & Gorman, 1974). In those cases the conductance changes decay within at least several seconds, but it appears that the time course may depend on the experimental procedures. In the snail neurone, Ca^{2+} injection or repetitive depolarization cause changes that last many seconds. In the same preparation there is substantial recovery after 250 msec, when a single brief depolarizing pulse is used (Meech & Standen, 1975). In the barnacle photoreceptor the apparent time course is less than 1 sec. Some authors have suggested that the duration of the increased $[Ca^{2+}]_i$ is

controlled by a metabolically driven Ca^{2+} pump, mainly in the mitochondria (Krnjević & Lisiewicz, 1972; Rose & Loewenstein; Hanani & Hillman, 1976). In view of the very high concentration of mitochondria in the barnacle photoreceptor (Fahrenbach, 1965) the fast recovery in this preparation may be due to a more rapid Ca^{2+} uptake by the mitochondria. Light-induced changes in $[Ca^{2+}]_i$ in barnacle and *Limulus* photoreceptors were measured by Brown & Blinks (1974), using aequorin. These measurements show a peak in $[Ca^{2+}]_i$ a few hundred msec after the onset of light, which declines to a low level in about 1 sec. According to the current views of light-adaptation in invertebrate photoreceptors, intense light increases $[Ca^{2+}]_i$ (Brown & Lisman, 1975; Hanani & Hillman, 1976). In the case of the barnacle photoreceptor this increase is presumably largely a result of Ca^{2+} influx, and this is in agreement with our results on the influence of low $[Ca^{2+}]_o$ on the dip (Fig. 3). The effects of light- and dark-adaptation are also pertinent to this point (Fig. 2), as dark-adaptation presumably involves the lowering of $[Ca^{2+}]_i$. We suggest that in the dark-adapted cell or in the low Ca^{2+} saline light flashes fail to increase $[Ca^{2+}]_i$ to the level needed to produce an effect. This level may be rather high, since in the snail neurone relatively large amounts of Ca^{2+} have to be injected in order to produce changes in membrane resistance (Meech, 1974).

Lisman & Brown (1972) found that increased $[Ca^{2+}]_i$ reduces the light-induced current in the ventral eye of *Limulus* and Hagins (1972) proposed that Ca^{2+} decreases the dark current in vertebrate photoreceptors; in both cases the current is carried mainly by Na^+. In principle, such a mechanism could account for our observations: a transient suppression of Na^+ current caused by a transient increase in Ca^{2+} would generate a negative-going phase in the response. However, the strong dependence of the dip on K^+, its reversal around membrane potential of -80 mV, and the effects of K^+ blockers, suggest that the main cause of the dip is not a Na^+ conductance decrease.

When the membrane is depolarized above -20 mV the dip starts to diminish until it disappears together with the whole response. From our results (Fig. 3), an influx of Ca^{2+} seems to be required to initiate an increase in P_K. The decrease of the dip at high membrane potentials may possibly be attributed to moving towards the Ca^{2+} equilibrium potential but there may be other possible explanations, which cannot be ruled out by the available experimental data.

The permeability changes discussed above may have a considerable functional significance. It is known that light-adaptation improves the temporal resolution in invertebrate photoreceptors (Fuortes & Hodgkin, 1964; Dodge, Knight & Toyoda, 1968). Increased $[Ca^{2+}]_i$ mimics this

effect (Brown & Lisman, 1975), but although the authors refer only to the shortening of the latency, it is evident from their recordings that the time course of the response also changes, showing faster decay after light-adaptation or intracellular Ca^{2+} injection. No mechanism for the action of Ca^{2+} on the response has been suggested. Our results indicate that changes in P_K may be involved. We propose that increased P_K induced by high Ca^{2+} may be an important factor in changing the temporal characteristics of the response. Intense light raises Ca^{2+}, which in turn increases P_K. This tends to shift membrane potential towards K^+ equilibrium potential and thus shortens the duration of the response.

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