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Single-channel recordings demonstrate that cGMP opens the light-sensitive ion channel of the rod photoreceptor

(retina/sensory transduction/second messengers/ion channels)

GARY MATTHEWS

Department of Neurobiology and Behavior, The State University of New York, Stony Brook, NY 11794

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ABSTRACT **Patch-clamp recordings were made from outer segments of single dark-adapted rod photoreceptors from toad retina. When patch pipettes were filled with a solution free of divalent cations, inward current through individual light-sensitive ion channels was sufficiently large to allow single-channel recording. Channel activity was suppressed by illumination within the normal response range of the dark-adapted rod. When illumination was restricted to a portion of the outer segment, the effect of light on channel activity was spatially localized to the illuminated region. Hyperpolarization of the recorded patch did not reduce the frequency of channel opening, indicating that the suppression of channel activity by light was due to illumination itself and not to light-induced hyperpolarization of the rod. After recording single light-sensitive channel activity in the intact cell, the patch of membrane was detached to form an excised, inside-out patch. Cyclic GMP applied to the intracellular face of the excised patch then opened a channel that appeared to be the same as the light-sensitive channel recorded earlier from the same membrane in the intact, functioning rod. This provides direct evidence that cyclic GMP acts as an internal transmitter that opens the light-sensitive channel of the vertebrate photoreceptor.**

Absorption of light by a rod photoreceptor hyperpolarizes the cell by closing cation channels in the plasma membrane. This process requires an intracellular transmitter to carry the signal from the light-absorbing pigment in the membrane of intracellular disks to the ion channels in the plasma membrane. Guanosine 3′,5′-cyclic monophosphate (cGMP) has generated considerable interest as a candidate for the intracellular transmitter of visual transduction. Light rapidly reduces the concentration of cGMP via a light-activated cGMP phosphodiesterase (1, 2), and recent reports show that cGMP activates a cationic conductance in excised patches of rod membrane (3–8). This suggests that cGMP keeps the light-sensitive channel open in darkness, and that the channels close in light as the concentration of cGMP inside the rod decreases. However, direct linkage between the channel closed by light in the intact rod and the channel opened by cGMP in excised membranes has not been demonstrated. Noise analysis suggests that the two conductances are kinetically similar (5, 7), but comparison has been hampered by the fact that the conductance of the light-sensitive channel is too small under physiological conditions to allow resolution of channel activity at the single-channel level (9–11), possibly because of channel blockade by divalent cations (5, 7, 9). In this paper, I report that single-channel recordings can be obtained from the light-sensitive channel of intact, dark-adapted rod photoreceptors when divalent cations are omitted from the solution bathing the external membrane face. This strategy is the same as that used recently to observe single cGMP-activated channels in excised patches of outer segment membrane (12–14).

After recording channel activity in the intact cell, the patch of membrane was excised, allowing access to the intracellular face of the membrane. Application of cGMP to the intracellular face of the excised patch then opened a channel that appeared identical to the light-sensitive channel recorded earlier in the same membrane patch. This provides direct evidence that cGMP is the internal transmitter controlling the light-sensitive channel of the rod photoreceptor.

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MATERIALS AND METHODS

Cell-attached and cell-free patch-clamp recordings were made from the outer segments of isolated rod photoreceptors by using standard techniques (15). As described (16), rods were isolated from toad retina (*Bufo marinus*) and placed on the stage of a compound microscope equipped for infrared viewing. Patch pipettes were filled with modified Ringer's solution (0-Ca^{2+}/Mg^{2+} Ringer's solution) containing 118 mM NaCl, 2.5 mM KCl, 10 mM Hepes, 0.15 mM EDTA, and no added Ca^{2+} or Mg^{2+}, pH 7.8. Omission of divalent cations allowed resolution of the light-sensitive conductance at the single-channel level. The solution bathing the cells was normal toad Ringer's solution (16).

In experiments on excised patches, cGMP (1–10 μM) was applied to the inner face of the membrane by local perfusion. The perfusate was the same 0-Ca^{2+}/Mg^{2+} Ringer's solution used to fill the patch pipettes.

RESULTS

Inward current through the cGMP-dependent conductance of excised patches of photoreceptor membrane increases dramatically when divalent cations are removed from the solution bathing the extracellular membrane face, suggesting that divalent cations act as channel blockers (7, 8, 17). If the light-sensitive conductance of intact rods behaves similarly, removing external divalent cations might increase the inward current through single light-sensitive channels sufficiently to allow single-channel recordings. To examine this, cell-attached patch-clamp recordings were made from intact, dark-adapted toad rods using patch pipettes filled with 0-Ca^{2+}/Mg^{2+} Ringer's solution. In ≈50% of experiments of this kind, brief inward current transients were observed in darkness; illumination reduced the frequency of these events, and this response was graded with light intensity over the normal response range of the dark-adapted rod. Results from an experiment of this kind are shown in Fig. 1. As expected from the fact that the internal transmitter spreads longitudinally only a few micrometers from an illuminated region (18, 19), only light centered on the patch was effective in reducing channel activity (Fig. 1*A Left*). Light placed 37 μm away along the outer segment was without effect (Fig. 1*A Right*). Because the light-induced hyperpolarization would be com-

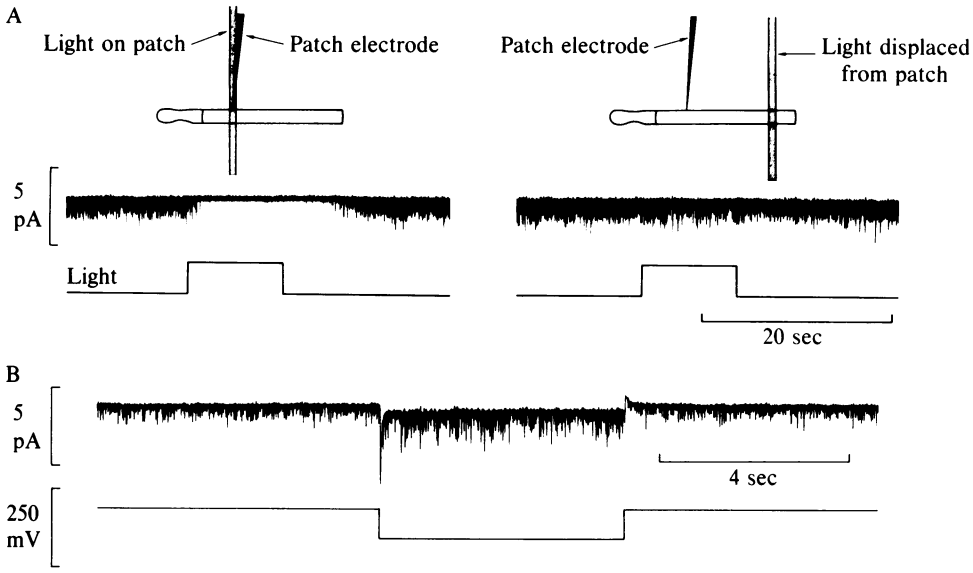

FIG. 1. Light-sensitive channel activity in a cell-attached patch from the outer segment of a dark-adapted rod photoreceptor. (*A*) Suppression of channel activity by light centered on the recorded patch (*Left*) but not by light displaced 37 μm toward the tip of the outer segment (*Right*). Diagrams (*A Upper*) show light placement and were traced from the TV screen during the experiment. Light was restricted to a narrow transverse slit (shaded region), and the direction of light propagation was perpendicular to the plane of the page. Nominal slit width was 3.4 μm; light intensity, 300 photons $\mu m^{-2} \cdot sec^{-1}$; wavelength, 500 nm. (*A Lower*) The upper trace shows the membrane current (inward negative; bandwidth, 0–80 Hz), and the lower trace shows the duration of light stimulus given by the upward step. Seal resistance was 25 GΩ. (*B*) Lack of effect of patch polarization on the rate of channel opening. During the downward step, pipette potential was stepped from 0 mV (i.e., the same potential as that of the bath) to +95 mV, thereby hyperpolarizing the patch by 95 mV. The upper trace shows the patch membrane current (bandwidth, 0–80 Hz), and the bottom trace shows the change in patch potential.

parable for both stimulus placements, this result suggests that the reduction in channel activity was caused by illumination *per se*, rather than by the resulting hyperpolarization.

The effect of membrane potential on channel activity was tested directly by varying the holding potential of the pipette interior. If light-induced hyperpolarization were responsible for reduction in channel activity during illumination, the rate of channel opening should decrease during a hyperpolarizing voltage step. However, the rate of channel activity was largely unaffected by hyperpolarizing the patch (Fig. 1*B*), confirming that the abolition of channel activity by light was not due to hyperpolarization.

Higher resolution recordings of light-sensitive channel activity at two holding potentials are shown in Fig. 2. The channel appeared to flicker rapidly in the open state, so that the variance of the current during channel events was greater than the background variance in the absence of channel activity. Flicker of the light-sensitive channel was suggested earlier by noise measurements (7, 20). This behavior of the channel is reminiscent of "flickery block" observed in other ionic channels (21, 22). Whether the flicker results from a blocking particle entering the channel or from an intrinsic property of the channel is unclear at present. Flicker that was unresolved because of limited recording bandwidth likely

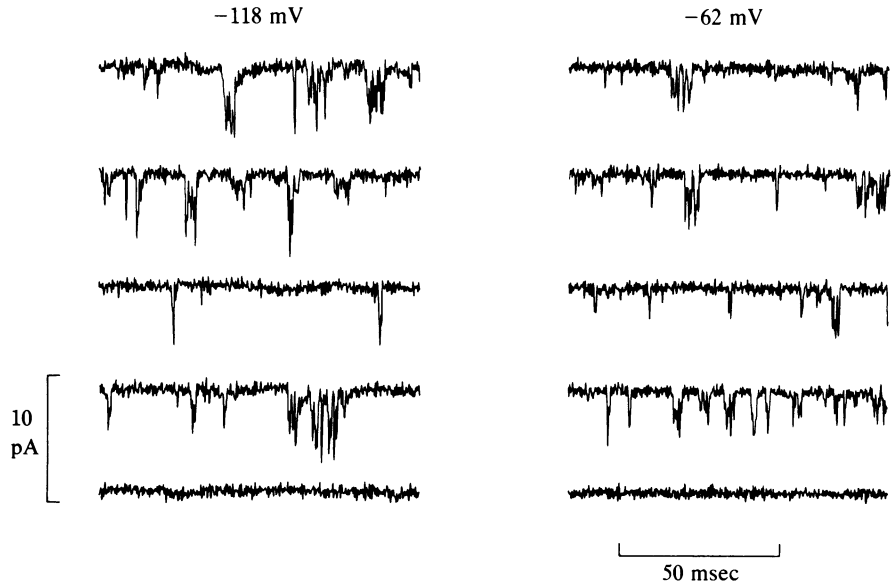

FIG. 2. Higher resolution recordings of light-sensitive channel activity at two holding potentials (same experiment as in Fig. 1). The bottom trace at each potential is in bright light, and the other traces are in darkness. Numbers at the top give the change in membrane potential from the resting dark level. Bandwidth, 0–2000 Hz.

accounts for at least part of the apparent variation in amplitude of the channel events in Fig. 2, particularly for brief events.

In six experiments, after characterization of the light-sensitive channel in the intact rod, the patch of membrane was pulled off from the outer segment in the inside-out configuration (15). cGMP was then applied to the exposed intracellular face. Individual cGMP-sensitive channel events (12–14) could be observed in the excised patch, as illustrated in Fig. 3*B*. The cGMP-sensitive channel events appeared indistinguishable, including flicker in the open state, from the light-sensitive channel recorded earlier in the same patch of membrane (compare *A* and *B* in Fig. 3). As an overall comparison of the shape of the light-sensitive and cGMP-sensitive channel events, power spectral densities were calculated from unselected, random samples of channel activity by subtracting the spectrum in bright light or in the absence of cGMP from the spectrum in darkness or in the presence of cGMP. In agreement with the impression from visual inspection, the two spectra were superimposable (Fig. 3*C*).

Because of unresolved flicker, it was difficult to arrive at an unambiguous estimate of single-channel current. To compare the amplitudes of the events, probability density functions of current amplitude were measured for light-sup-

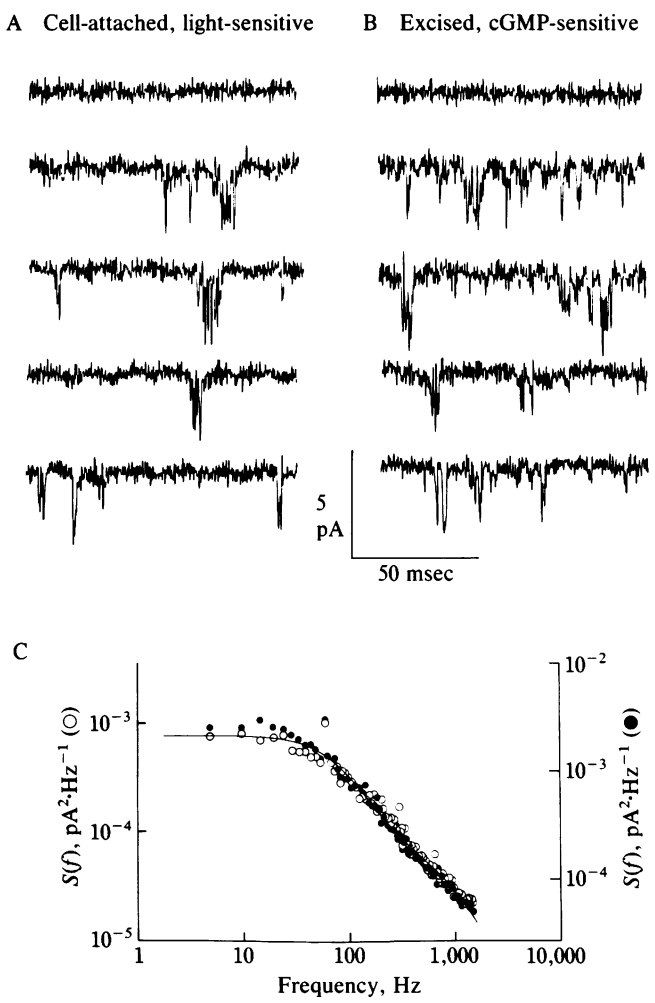

FIG. 3. Light-sensitive and cGMP-sensitive channel activity in the same patch of outer segment membrane before and after excising the patch from the rod. (*A*) Light-sensitive channel activity recorded in the cell-attached mode from intact rod. The top trace is in bright light, and the others are in darkness. The patch was hyperpolarized by 100 mV. Seal resistance was 20 GΩ. (*B*) cGMP-sensitive channel activity in the same patch after detachment from the rod in the inside-out configuration. The inner membrane face was perfused with 0-Ca^{2+}/Mg^{2+} saline. The top trace is in the absence of cGMP, and the others are with cGMP on the intracellular face. cGMP was applied and removed by moving the patch pipette between two streams of perfusate, one with 10 μM cGMP and the other without cGMP, emerging from a pair of perfusion micropipettes placed about 50 μm apart. Because of mixing between the two streams, the actual concentration of cGMP at the patch electrode was <10 μM. The patch membrane potential was −87 mV. (*C*) Power spectral densities, $S(f)$, of light-sensitive (○) and cGMP-sensitive (●) channel activity. Baseline spectra in bright light or in the absence of cGMP were subtracted. Spectra were calculated from 127 (light-sensitive) or 73 (cGMP-sensitive) 0.2-sec samples of channel activity, digitized at 0.1-msec intervals with a bandwidth of 0–2000 Hz. Spectra were calculated and plotted as described (23). Because of the higher frequency of channel events in the excised patch, the cGMP spectrum was shifted downward by 0.4 logarithmic unit to superimpose with the light-sensitive spectrum. The solid line through data points was drawn according to a sum of two Lorentzian equations, with corner frequencies of 80 and 960 Hz and with a zero-frequency asymptote of the high-frequency component that was 0.07 that of the low-frequency component.

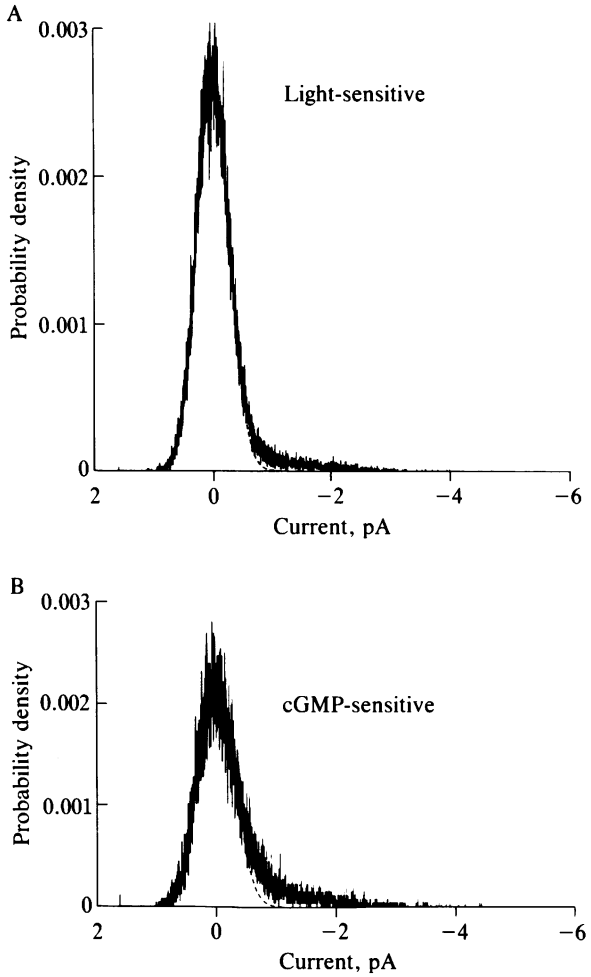

FIG. 4. Probability density functions of the current amplitude for light-sensitive (*A*) and cGMP-sensitive (*B*) channel activity. The same patch as in Fig. 3 was used. Dashed lines show Gaussian probability density functions with standard deviations of 0.27 pA (*A*) or 0.29 pA (*B*) fitted to background fluctuations in the absence of channel activity (peak at 0). In *A* the cell-attached patch was hyperpolarized by 100 mV from the dark potential, for a total driving force of about 140–150 mV, and in *B* the membrane potential of the excised patch was −87 mV. The tail of inward (negative) current corresponding to channel openings was comparable in *A* and *B*, indicating that the amplitude of channel current was about the same in the cell-attached and excised-patch recordings despite the difference in driving force in the two conditions.

pressed and cGMP-activated channel activity. As shown in Fig. 4, probability density functions for the two types of channel activity were similar for the cell of Fig. 3, confirming the visual impression (compare events in *A* and *B* of Fig. 3) that the light-sensitive and cGMP-activated channel events were of similar amplitude. Although the amplitudes were similar, the driving force for the current was greater for the cell-attached recording (about 140–150 mV, assuming a resting driving force of 40–50 mV) than for the excised patch (90 mV). This suggests that the conductance of the channel in the excised patch was larger than in the intact cell. A higher conductance in the excised patch might occur, for example, if the divalent cation concentration on the intracellular face of the excised patch (0-Ca^{2+}/Mg^{2+} Ringer's solution) were lower than in the intact rod.

DISCUSSION

Previous work has suggested that the cGMP-sensitive conductance of rod photoreceptor membranes is similar in a number of ways to the light-sensitive conductance of the intact cell. Noise analysis demonstrated similar kinetics for light-sensitive and cGMP-sensitive channels (5, 7), and the current–voltage relations of both conductances show pronounced outward rectification under physiological ionic conditions (4, 7, 8). Yau and Nakatani (24) showed that the effect of exogenously supplied cGMP on the conductance of a perfused outer segment could be counteracted by light, and Zimmerman *et al.* (25) found that relatively nonhydrolyzable analogs of cGMP had similar actions on the cGMP-sensitive and light-sensitive conductances. The result reported here establishes a direct link between the cGMP-sensitive channel of excised rod membrane and the light-sensitive channel of the intact rod; the channel that is closed by light in the intact cell appears to be opened by cGMP in the excised patch.

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