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PROPERTIES OF ION CHANNELS CLOSED BY LIGHT AND OPENED BY GUANOSINE 3′,5′-CYCLIC MONOPHOSPHATE IN TOAD RETINAL RODS

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SUMMARY

1. In patch-clamp recordings from outer segments of dark-adapted rod photoreceptors, single-channel recordings were obtained from the light-sensitive conductance when divalent cations were omitted from the pipette solution bathing the extracellular face of the recorded patch of membrane.

2. Activity of the light-sensitive channel was suppressed by light within the normal response range of the dark-adapted rod. During dim, steady illumination, the rate of opening of the channel fluctuated dramatically, as expected qualitatively from statistical fluctuations in the number of photoisomerizations occurring within the effective collecting area of the recorded patch.

3. The light-sensitive channel flickered rapidly in the open state, so that individual events appeared as a burst of openings and closings. The average duration of a burst was $0{\cdot}78 \pm 0{\cdot}03$ ms (mean $\pm$ S.E.). The average duration of an individual opening was $0{\cdot}18 \pm 0{\cdot}008$ ms. The average closed duration within a burst was $0{\cdot}37 \pm 0{\cdot}02$ ms.

4. Hyperpolarization of the recorded patch had no effect on average burst or open duration, although opening frequency increased slightly ($+18{\cdot}6 \pm 4{\cdot}9\%$; $n = 13$; mean $\pm$ S.E.). Average single-channel current increased linearly with hyperpolarization, giving an estimated single-channel conductance of $20{\cdot}5 \pm 1{\cdot}1$ pS. By extrapolation of the relation between channel current and hyperpolarization, the dark driving force was estimated to be about 48 mV.

5. In addition to reducing the rate of channel events, dim non-saturating light also reduced the average duration of a burst of openings and the average duration of openings within a burst.

6. About 50% of cell-attached patches showed no channel activity in darkness. Light-suppressable channel activity could be induced in these silent patches by perfusing the outer segment with low-Ca^{2+} Ringer solution. Similarly, activity could be increased dramatically by low-Ca^{2+} Ringer solution in patches that did show channel activity in the dark. From the maximal channel activity observed during low-Ca^{2+} perfusion, the lower limit for the number of channels per patch was 20–70, corresponding to an estimated channel density of 100–350 channels μm^{-2}.

7. After recording light-sensitive channel activity in the intact rod, the patch of membrane was excised, exposing the intracellular membrane face. Application of

guanosine 3′,5′-cyclic monophosphate (cyclic GMP) to the intracellular face activated channels (Haynes, Kay & Yau, 1986; Zimmerman & Baylor, 1986; Matthews, 1986*d*, 1987) whose properties could then be compared directly with the light-sensitive channels recorded earlier in the same patch of membrane.

8. Channels activated by cyclic GMP were indistinguishable in the following ways from the light-sensitive channel recorded in the same patch: flicker in the open state, burst duration, open duration, closed duration within a burst, probability density function of current amplitude, and effect of membrane potential on temporal properties of the channel. The estimated single-channel conductance was slightly higher on average in the excised patch (24·0 *vs.* 20·5 pS); this difference might reflect differences between intracellular fluid and the modified Ringer solution used on the intracellular face of excised patches.

9. The results demonstrate that the channel opened by cyclic GMP in excised patches is the same as the light-sensitive channel of the intact rod. Taken together with previous biochemical evidence, this provides strong indication that cyclic GMP is the internal transmitter responsible for modulation of plasma membrane conductance by light in the rod photoreceptor.

INTRODUCTION

Light hyperpolarizes vertebrate photoreceptors by reducing a steady inward current that enters the outer segment in darkness (Hagins, Penn & Yoshikami, 1970; Baylor, Lamb & Yau, 1979*a*). Electrical measurements have contributed greatly to our understanding of this light-sensitive conductance mechanism, but resolution of the conductance at the single-channel level has remained elusive. Because of the small size of the single-channel current (< 10 fA), noise analysis has been the only means available to gather information about the size and kinetics of the unitary conductance events underlying the light-sensitive current (Bodoia & Detwiler, 1984*a*, *b*; Gray & Attwell, 1985; Zimmerman & Baylor, 1985; Matthews, 1985*a*, *b*, 1986*a*). However, there is evidence that external Ca^{2+} blocks the light-sensitive channel (Bodoia & Detwiler, 1984*b*; Matthews, 1985*b*), which suggests that removal of divalent cations from the extracellular fluid might increase the single-channel current sufficiently to allow resolution of single channels. In addition, recent data show that Ca^{2+} and Mg^{2+} reduce the amplitude of the cyclic GMP-activated conductance of excised patches of rod membrane (Yau & Haynes, 1986; Matthews, 1986*a*; Haynes, Kay & Yau, 1986; Zimmerman & Baylor, 1986) and that removal of divalent cations permits single-channel recordings from the cyclic GMP-sensitive conductance (Haynes, Kay & Yau, 1986; Zimmerman & Baylor, 1986; Matthews, 1986*d*, 1987). In the experiments reported here, cell-attached patch-clamp recordings were made from intact, dark-adapted rod photoreceptors using patch pipettes filled with Ringer solution lacking divalent cations. Openings of individual light-sensitive channels could be resolved in these recordings, and a major portion of this paper will examine the properties of the light-sensitive conductance revealed by these single-channel recordings.

Additionally, after recording light-sensitive channel activity in the intact rod, patches were detached in inside-out configuration in order to allow activation of the

cyclic GMP-sensitive conductance by application of cyclic GMP to the intracellular membrane face (Fesenko, Koleshnikov & Lyubarsky, 1985). This provided a direct comparison of the light-sensitive and cyclic GMP-activated channel in the same patch of membrane. Individual cyclic GMP-activated channel events were indistinguishable in a number of ways from light-sensitive channel events recorded earlier in the same membrane. This establishes a direct link between the channel closed by light in the intact rod and the channel opened by cyclic GMP in excised membranes.

An abstract (Matthews, 1986*d*) and a brief preliminary report (Matthews, 1987) of some of the results presented here have appeared.

METHODS

Animals and preparation of retina

Toads, *Bufo marinus*, were obtained from commercial suppliers and maintained in a large, inclined tank with water at one end. At the other end was a dry platform warmed by a heat lamp. Twice each week animals were fed with semi-dry cat food (Purina Tender Vittles), the tank was cleaned, and the water was exchanged. The light cycle was 12/12 h light/dark, and recordings were made during the light portion of the cycle. Before an experiment, animals were dark-adapted overnight.

All recordings were made from isolated rod photoreceptors obtained by mechanical dissociation of isolated retina as described previously (Baylor *et al.* 1979*a*; Hodgkin, McNaughton, Nunn & Yau, 1984). Under dim red light, a toad was killed by pithing the brain and spinal cord, and one eye was removed and hemisected. The second eye was left in the pithed carcass and used 3–6 h later. Under infra-red illumination, a section of retina was peeled away from the back of the eye and placed photoreceptor side up in a dish lined with silicone elastomer (General Electric, RTV615). The dish was filled with Ringer solution containing (mM): NaCl, 111; KCl, 2·5; $CaCl_2$, 1·0; $MgCl_2$, 1·6; glucose, 10; HEPES, 10; pH = 7·8. The section of retina was then minced into small pieces with a razor blade. A drop containing a slurry of minced pieces was transferred to the recording chamber, and the chamber was filled with Ringer solution (volume = 0·6 ml). The recording chamber was placed on the stage of a compound inverted microscope equipped with an infra-red-sensitive TV system. The chamber had a glass top and bottom to provide an optically advantageous path for high-magnification viewing of cells and electrodes, and access for entry of electrodes into the chamber was through the open front. Fluid was held in the chamber by surface tension.

Electrodes and recording apparatus

Patch pipettes were pulled from Pyrex tubing (o.d. = 1·2 mm; i.d. = 0·6 mm) and had outer tip diameters of 0·7–1·4 μm before fire-polishing. When filled with saline, electrodes typically had resistances of 30–40 MΩ. In some experiments, electrode shanks were coated with GE RTV615 silicone elastomer. Electrodes were filled with 0 Ca^{2+}, 0 Mg^{2+} Ringer solution containing (mM): NaCl, 118; KCl, 2·5; HEPES, 10; EDTA, 0·15, pH = 7·8. The bath electrode in the chamber was a sintered Ag–AgCl pellet, and the patch pipette electrical connection was via a chlorided silver wire. The output of the patch-clamp amplifier (List L/M-EPC7) was recorded on an FM tape-recorder (Racal Store 4DS) at band width 0–5000 or 0–10000 Hz. Light monitor output, voltage-clamp command steps, and synchronizing pulses were also recorded. For computer analysis, selected segments were replayed through an anti-aliasing, low-pass filter and digitized in a PDP 11/73 computer. For power spectral analysis, the anti-aliasing filter was a 6-pole Butterworth, usually set to 2 kHz cut-off frequency, and the sampling rate was 5000–10000 s^{-1}. For analysis of channel events, an 8-pole Bessel filter, usually set to 4 kHz, was used, and the sampling rate was 20000–40000 s^{-1}.

Light stimuli

Illumination was controlled by a dual-beam optical stimulator similar to that described by Baylor & Hodgkin (1973). One beam supplied infra-red illumination ($\lambda > 850$ nm; Schott RG850

glass filter) for viewing manipulations, and the other beam supplied 500 nm light (interference filter; half band width = 10 nm). Light intensity was controlled by neutral density filters. In most experiments, diffuse illumination was used (spot diameter = 600 μm). In experiments using slit stimuli, an adjustable monochromator slit was placed in the optical path at the position of the field lens, and an image of the slit was focused on the recorded cell using the microscope condenser. At the end of each experiment, intensity of the 500 nm light was calibrated by placing a calibrated photometer sensor on the stage of the microscope at the position of the recording chamber.

Recording procedure

Manipulations were viewed on the screen of a video monitor fitted with a red safelight filter. Isolated rod photoreceptors were located among detached outer segments and pieces of minced retina on the floor of the chamber and appeared to be intact except for the synaptic terminal. Under visual control, the patch pipette was brought in perpendicular to the long axis of the rod until it just touched the side of the outer segment. Gentle suction was then applied by mouth to the pipette interior, causing a slight sideways movement of the cell against the pipette. In successful recordings, this sideways jump was accompanied by immediate formation of a gigaohm seal. Suction was released immediately upon seal formation; there was no visible entry of material into the pipette and little or no change in the apparent diameter of the cell at the point of contact. Seal resistances ranged from 1–100 GΩ, although in most experiments the range was 10–30 GΩ.

To form an excised inside-out patch, the electrode was pulled back slightly from the cell, and the micromanipulator was abruptly tapped. Formation of sealed vesicles rather than inside-out patches was a common complication. Incidence of vesicle formation appeared to be reduced by local perfusion of the outer segment with 0 Ca^{2+}, 0 Mg^{2+} Ringer solution before excising the patch. Also, vesicles could sometimes be opened by sharply tapping the micromanipulator while perfusing the tip of the patch pipette with 0 Ca^{2+}, 0 Mg^{2+} Ringer solution, by rubbing the pipette tip against a small piece of silicone elastomer, or by briefly moving the pipette through the air–water interface at the front of the recording chamber. Outside-out patches were formed by first rupturing the patch of membrane within the pipette to enter the whole-cell recording mode (Hamill, Marty, Neher, Sakmann & Sigworth, 1981), then pulling the electrode back from the cell. Rupture of the cell-attached patch was often a spontaneous, and unwelcome, occurrence.

Perfusion

Solutions were applied to excised patches by local perfusion (Liebman, Mueller & Pugh, 1984). A pair of perfusion pipettes with orifice diameters of approximately 8 μm were placed side-by-side near the patch pipette. Cyclic GMP was applied to inside-out patches by moving the patch electrode between the two parallel streams of perfusate, one with cyclic GMP and the other without cyclic GMP, emerging from the perfusion pipettes. The concentration of cyclic GMP ranged from 5 μM to 1·25 mM in different experiments. The perfusate was the same 0 Ca^{2+}, 0 Mg^{2+} Ringer solution used to fill the patch pipette. This was done so that (1) conditions would be comparable to previous experiments using noise analysis (Matthews, 1986*a*) and (2) rectification properties of the channels could be studied with symmetrical ion concentrations on the two sides of the membrane.

Data analysis

Power spectral analysis was performed as described by Baylor, Matthews & Yau, (1980). Analysis of single-channel properties was performed on digitized samples of channel activity. To define the transition between open and closed states, a half-amplitude criterion was used (Colquhoun & Sigworth, 1983). The criterion was set using selected samples that had long channel events with particularly little flicker. This criterion was then used for all other samples. Samples that had overlapping opening of two or more channels were excluded from the analysis. Average current in the open state was estimated by averaging all values that exceeded the criterion and taking the difference between this average and the base line. For display and plotting of channel activity, values between the actually sampled data points were estimated using a cubic-spline interpolation (Colquhoun & Sigworth, 1983).

Exponential functions were fitted to semi-logarithmic plots of distributions of channel durations by minimizing the sum over all data points of the square of the orthogonal distance of the point from the line. In the fitting, the first one or two bins were ignored because of limited band width of the recording apparatus.

RESULTS

Resolution of the light-sensitive conductance at the single-channel level

The results will be presented in two major sections. In this section, the general properties of the light-sensitive channel will be discussed. In the second section, the cyclic GMP-activated channel in excised membrane patches will be compared with the light-sensitive channel recorded in the same patch before it was detached from the dark-adapted rod.

Channels closed by light in intact, dark-adapted rods

Removal of external divalent cations revealed light-sensitive single-channel activity. When divalent cations are removed from the solution bathing the external face of the membrane, inward current can be passed more readily by the cyclic GMP-activated conductance of excised outer segment patches (Yau & Haynes, 1986; Haynes, Kay & Yau, 1986; Zimmerman & Baylor, 1986; Matthews, 1986*a*), and it is possible to record openings of individual cyclic GMP-activated channels (Haynes *et al.* 1986; Zimmerman & Baylor, 1986; also, see below). If the light-sensitive conductance of intact rods behaves similarly, then 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+}, 0 Mg^{2+} Ringer solution. Three types of result were observed in these recordings: (1) no light-dependent activity; (2) large, light-suppressable noise that appeared to be made up of superimposed activity of a number of channels carrying inward current; and (3) discrete, inward-current channel events that were suppressed by light. Recordings yielding discrete channel activity (approximately 50% of all recordings) will be the principal focus of this paper.

Fluctuations in response to dim light. The effect of illumination on channel activity is shown in Fig. 1. With brighter light (top trace), all channel opening was abolished, leaving only a flat base line. The light intensity required to abolish channel activity produced about 200 photoisomerizations s^{-1} in the outer segment; this is comparable to the rate of isomerizations typically required to saturate the macroscopic light response of toad rods (e.g. Baylor, Lamb & Yau, 1979*b*). During dimmer steady illumination (bottom two traces), the response fluctuated markedly both across trials (compare middle and bottom traces in Fig. 1) and within a single light step. This behaviour is as expected from statistical fluctuation in the number of quanta absorbed within the effective photon collecting area of the recorded patch of membrane (Matthews, 1986*b*). Thus, the light-sensitive channel activity exhibits the high sensitivity and quantal fluctuations expected of the channels carrying the light-sensitive current of the rod photoreceptor.

Response to illumination is spatially restricted. Another prominent feature of phototransduction in rod photoreceptors is that the effect of illumination is spatially restricted, spreading only a few micrometres or less along the outer segment from an illuminated region (Lamb, McNaughton & Yau, 1981; Matthews, 1986*b*). To determine if the light-sensitive channel activity behaves similarly, light was restricted to a narrow, transversely oriented slit, which was centred on the recorded patch of

membrane or displaced longitudinally along the outer segment. When the slit was positioned 37 μm distant from the recorded patch, sensitivity was 1·3–1·6 log units lower than when illumination fell directly on the recorded region. Thus, the light-sensitive channels behaved in the manner expected of the channels responsible for the dark current of the dark-adapted rod. Because the reduction in sensitivity with

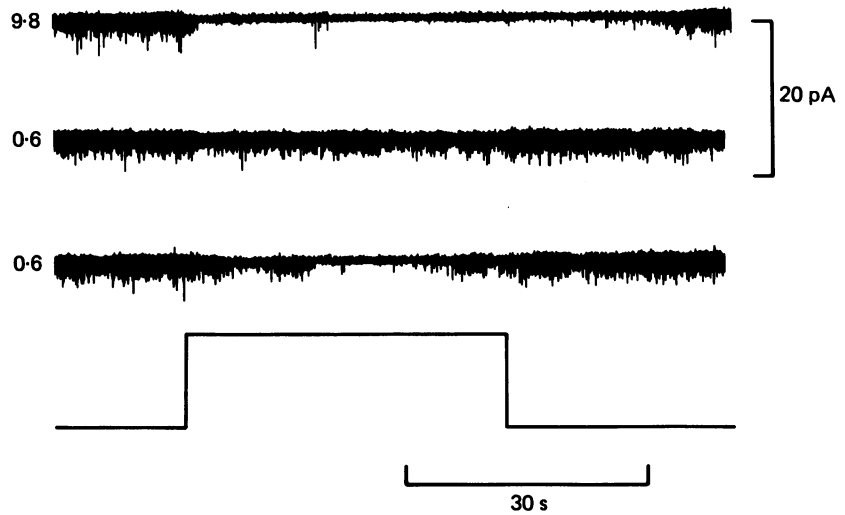

Fig. 1. Chart recorder traces showing effect of illumination on light-sensitive channel activity in a cell-attached patch of a dark-adapted rod. The timing of illumination is shown on the bottom trace, and the numbers to the left of each trace give the light intensity in photons $\mu m^{-2} s^{-1}$. Recording band width was limited by the chart recorder to 0–80 Hz. With brighter illumination (top trace), all channel activity was abolished by the light; the downward deflections midway through the light in the top trace are electrical artifacts caused by discharge of static electricity when the experimenter's hand touched part of the apparatus. With dimmer illumination, channel activity fluctuated considerably. Patch hyperpolarized by 115 mV.

displaced illumination was similar to the measured fall in light intensity at 30–40 μm from the slit (Matthews, 1985*c*), the response obtained with the longitudinally displaced slit was likely to be due to light scatter.

Rate of channel events. The frequency of light-sensitive channel events in darkness varied considerably across experiments, ranging from 0 (i.e. silent patches mentioned above) to 148 s^{-1}. In patches with activity, the average frequency was 55 ± 8 s^{-1} (mean $\pm$ S.E., $n = 17$). Some of this variation is attributable to differences in electrode tip configuration and thus to size of the recorded patch. However, apparently identically constructed electrodes could yield different rates of channel activity, suggesting that there might be regional variation in the density of active channels. Because patch area was unknown, this conclusion remains uncertain.

Kinetics of light-sensitive channel

Flicker in the open state. Higher resolution views of light-sensitive channel activity in six experiments are shown in Fig. 2. In every instance, the channel appeared to flicker rapidly in the open state, so that the variance of the current during events was

greater than the base-line variance. Such flicker was predicted in earlier experiments on the basis of the form of the power spectrum of light-sensitive current noise (Matthews, 1986*a*, *c*).

Definition of temporal parameters. Because of the brief closures (whether partial or complete) during a channel event, individual events typically appeared as groups of

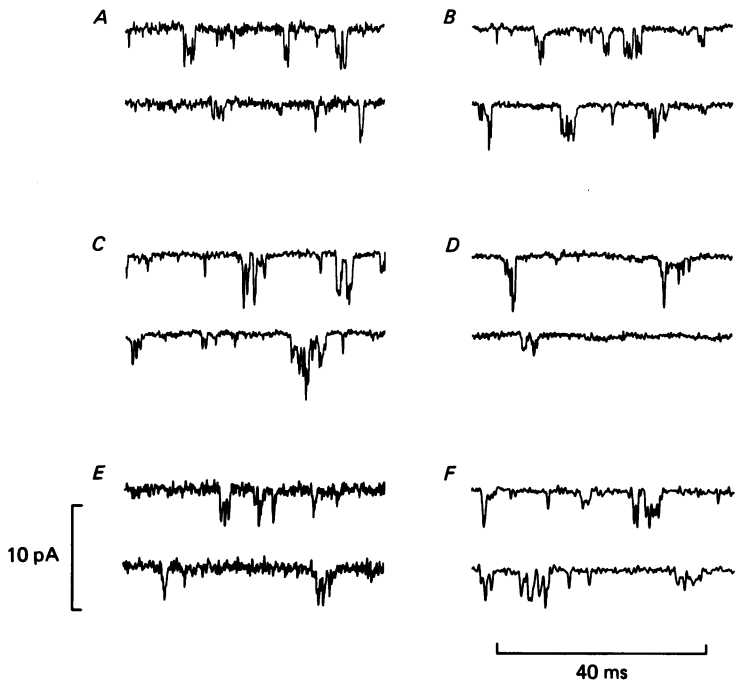

Fig. 2. High-resolution recordings of light-sensitive channel activity in six cell-attached recordings (*A–F*) from dark-adapted rods. Band width = 0–2000 Hz.

brief openings. Following the terminology of Neher & Steinbach (1978), such a group of brief openings will be called a burst. A burst was defined as starting with any opening, no matter how brief, that was separated from the last previous opening by a closed period of at least 1 ms. A burst ended with any closure of greater than 1 ms; that is, closures of less than 1 ms were ignored. Open–closed transitions were defined employing a half-amplitude criterion, as described in Methods. The other temporal parameters examined were the closed duration within a burst and the open duration. Again following Neher & Steinbach (1978), open duration refers to the duration of an individual excursion into the open state within a burst. That is, any transition below the open–closed criterion, no matter how brief, terminated an individual event in accumulating the distribution of open durations. Similarly, closed duration within a burst was defined as the duration of an individual excursion below the open–closed criterion. Note that because of the definition of a burst, closed durations within a burst effectively represent only closed events less than 1 ms in duration. Because the number of channels in each patch was unknown (but large; see below), the closed time between bursts is ambiguous and will not be discussed.

Burst duration. An example of the distribution of burst durations for light-sensitive

channel activity in a cell-attached recording is shown in Fig. 3 (circles). The triangles in Fig. 3 show results for cyclic GMP-activated channel activity, which will be discussed in a later section. Except for the briefest bin, the distribution up to a duration of 4 ms could be described by a single exponential, with a time constant of 0·94 ms. There were also occasional longer events, making up about 2% of the total

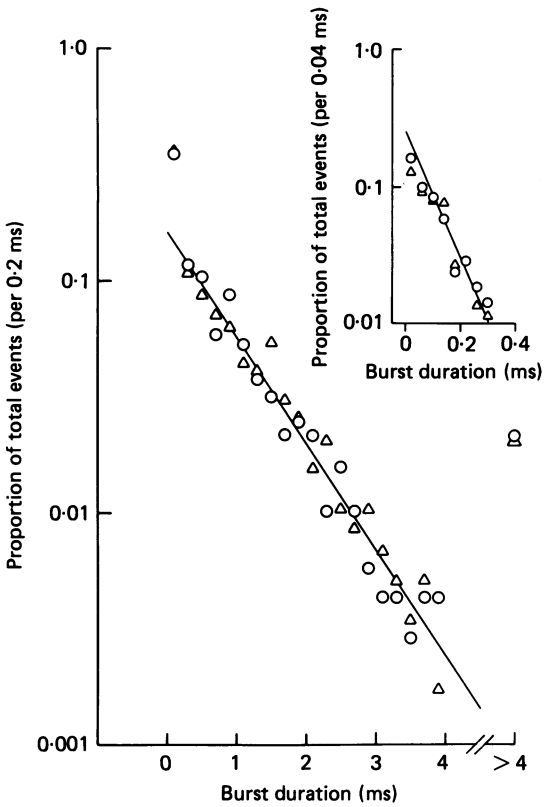

Fig. 3. Distribution of burst durations for light-sensitive (circles) and cyclic GMP-activated (triangles) channels in the same patch of outer segment membrane. Burst duration is defined in the text. The straight line was fitted to all bins less than 4 ms except the first and corresponds to an exponential time constant of 0·94 ms. The inset shows the first bin on an expanded scale. The straight line in the inset was fitted to the data ignoring the first two bins and corresponds to an exponential time constant of 0·1 ms. All burst durations greater than 4 ms were lumped together in the bin marked > 4.

number, that occurred significantly more frequently than expected from the single exponential distribution. It is not clear whether these longer events represent a separate kinetic state or two consecutive openings being counted as a single long burst because they were separated by less than the 1 ms closure criterion used to define termination of a burst. Brief bursts also occurred more frequently than expected from a single exponential. As shown on expanded time-scale in the inset of Fig. 3, the briefer events were distributed approximately exponentially, with a time constant of 0·1 ms. As shown in Table 1, burst duration of the light-sensitive channel averaged $0{\cdot}78 \pm 0{\cdot}03$ ms (mean $\pm$ S.E., seventeen experiments).

TABLE 1. Temporal parameters and single-channel conductance for light-sensitive and cyclic GMP-activated channels. Temporal parameters are defined in the text. Abbreviations: n, number of experiments; t_b, average burst duration; t_o, average open duration; t_c, average closed duration during a burst; γ, single-channel conductance. Numbers are means ±S.E.

	n	t_b (ms)	t_o (ms)	t_c (ms)	γ (pS)
Light-sensitive channel	17	0·78±0·03	0·18±0·008	0·37±0·02	20·5±1·07
Cyclic GMP-activated channel	11	0·85±0·07	0·23±0·01	0·38±0·03	24·0±1·19
Ratio (cyclic GMP-activated/light-sensitive)	7	1·09±0·08	1·12±0·08	1·01±0·06	1·35±0·08*

*$P < 0·01$ that true ratio is 1·0· 99% confidence interval estimated using t statistic (Hays, 1963).

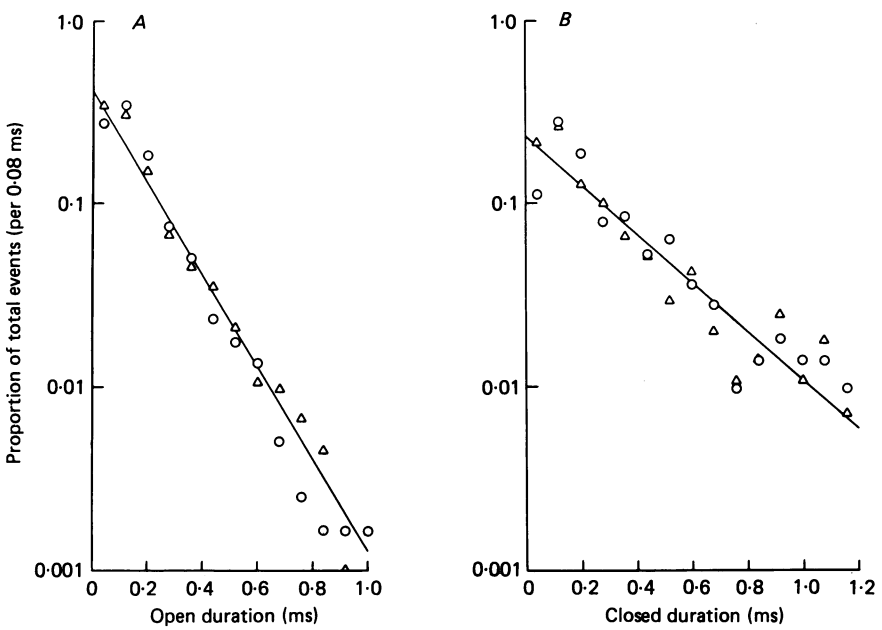

Fig. 4. Distributions of open durations (A) and closed durations within a burst (B) for light-sensitive (circles) and cyclic GMP-activated channels (triangles) within the same patch. Same experiment as Fig. 3. The straight lines correspond to exponential time constants of 0·17 and 0·33 ms for open and closed durations, respectively. The first two bins and all closed durations greater than 1 ms were ignored in fitting the straight lines to the data.

Open duration. Fig. 4A illustrates the distribution of open durations obtained by counting even brief closure as termination of the event (i.e. unlike the analysis of burst duration, no minimum closure was required). The circles in Fig. 4A show results from light-sensitive channel activity, and the triangles show results for cyclic GMP-activated channel activity, which will be discussed later. The distribution was fitted by a single exponential with time constant of 0·17 ms in this experiment. As shown in Table 1, open duration averaged 0·18±0·008 ms (mean±S.E.) in seventeen experiments.

Closed duration within a burst. The distribution of all closed durations less than 1 ms is shown in Fig. 4*B* (circles: light-sensitive; triangles: cyclic GMP-activated). This portion of the distribution was fitted by a single exponential with a time constant of 0·33 ms. In Table 1, the average value for closed durations less than 1 ms was 0·37 ± 0·02 ms (mean ± S.E.) in seventeen experiments.

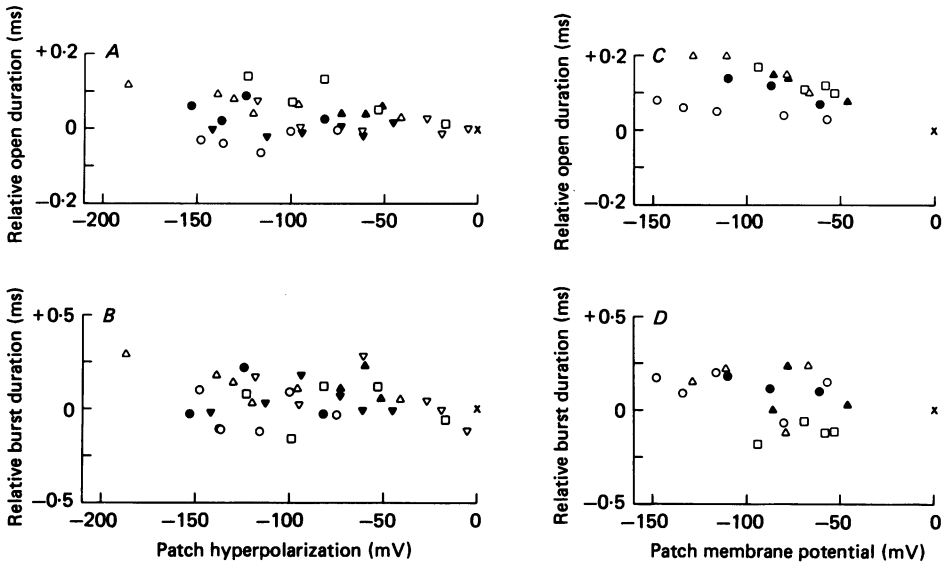

Fig. 5. Lack of effect of patch hyperpolarization on average open duration and average burst duration of the light-sensitive channel (*A* and *B*) and the cyclic GMP-activated channel (*C* and *D*). Each type of symbol represents results from a different experiment. In *A* and *B*, the average duration at 0 mV of hyperpolarization (i.e. the resting dark potential) was subtracted out; X on each graph marks this reference point. In *C* and *D*, data were normalized across experiments by subtracting the average duration at 0 mV, which was estimated for each experiment by extrapolating a straight line fitted to the observed data for that patch. X marks this extrapolated reference.

Effect of voltage on light-sensitive channel

Because the interior of the cell was not voltage clamped in these experiments, illumination hyperpolarized the rod and changed the membrane potential of the recorded patch. Therefore, examination of the effects of membrane voltage on channel activity is crucial to the demonstration that the abolition of channel activity by illumination is due to light *per se*, rather than to the resulting hyperpolarization. Indirect indication that light, rather than hyperpolarization, suppresses channel activity was obtained in the experiments using slit stimuli, discussed above. The hyperpolarizing effect of illumination should vary little with slit placement along the outer segment, yet light falling some distance away from the recorded region was more than 1 log unit less effective in suppressing channel activity than light falling directly on the recorded region.

A more direct test of voltage dependence is to hyperpolarize the recorded patch by making the pipette holding potential positive. Hyperpolarization by as much as

150 mV did not significantly reduce the rate of channel opening in darkness, confirming the expectation from the slit experiments. In thirteen experiments in which the patch was hyperpolarized by 25–100 mV, the average percentage change in event frequency was 18·6±4·9% (mean±S.E.; range, −6 to +52%). Thus, the frequency of opening in darkness was increased slightly by hyperpolarization, not

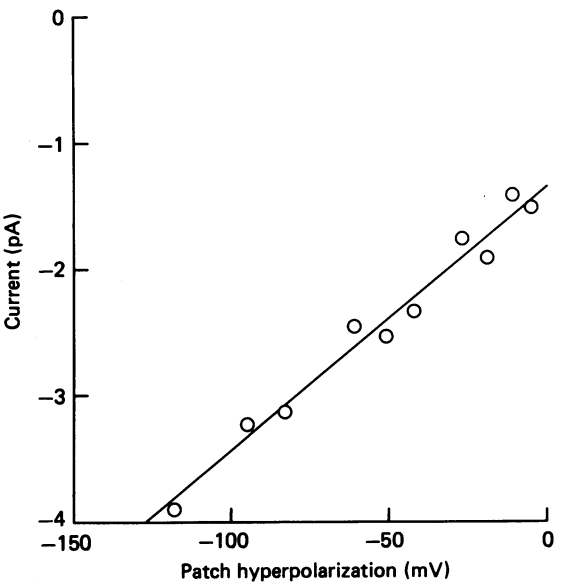

Fig. 6. Relation between average single-channel current and patch hyperpolarization for the light-sensitive channel. The straight line through the data corresponds to a single-channel conductance of 21·0 pS. Results from seventeen experiments of this kind are summarized in Table 1.

decreased as would be required if light-induced hyperpolarization rather than light itself was responsible for the reduction in channel opening in the presence of light.

Effect of voltage on temporal parameters. We examined the average open duration of the light-sensitive channel in darkness as a function of holding potential, and the results from seven experiments are summarized in Fig. 5*A*. In order to compare results across cells, all durations are expressed relative to the average open duration with no holding potential (i.e. with the pipette clamped to bath potential). The average duration was essentially unchanged by hyperpolarization, even to very negative potentials. In the seven experiments, a straight line was fitted to each cell's data, and the average slope of the lines corresponded to an average increase in open duration of 0·4±0·2 μs per millivolt of hyperpolarization (mean±S.E.). Results for average burst duration in the seven experiments are shown in Fig. 5*B*. Once again, hyperpolarization had little effect, increasing burst duration an average of 0·8±0·4 μs per millivolt of hyperpolarization (mean±S.E.).

Estimate of channel conductance. The single-channel conductance was estimated from the slope of the relation between channel current and patch hyperpolarization, as illustrated in Fig. 6. Samples that included superimposed openings of two channels were excluded from the analysis. In seventeen experiments, summarized in Table 1,

the channel conductance averaged 20·5 ± 1·07 pS (mean ± S.E.). In all experiments, the relation between current and voltage was approximately linear, as in Fig. 6. This suggests that with 0 Ca^{2+}, 0 Mg^{2+} Ringer solution on the external face of the membrane and intracellular fluid on the internal face, the current–voltage relation of the light-sensitive conductance (at least for inward current) was similar to that of the cyclic GMP-activated conductance with low concentrations of divalent cations on both sides of the membrane (Yau & Haynes, 1986; Haynes *et al.* 1986; Zimmerman & Baylor, 1986; Matthews, 1986*a*).

The linearity of the relation between hyperpolarization and single-channel current also permits the driving force of the current at the dark resting potential to be estimated by extrapolating the linear relation to zero current. In nine experiments with four or more data points, the estimated reversal point for the current through the light-sensitive channel was 48·2 ± 6·9 mV (mean ± S.E.) more positive than the dark resting potential. This agrees well with the 50 mV driving force that would be expected from a resting potential of about −40 mV and a reversal potential of approximately +10 mV (Bodoia & Detwiler, 1984*b*; Baylor & Nunn, 1986).

Effect of non-saturating illumination on temporal properties

The most dramatic effect of light on the light-sensitive channel was to reduce the frequency of channel opening (e.g. Fig. 1). To determine if light had other effects on channel temporal properties, we examined channel openings that occurred during non-saturating, steady light. As described above, during such illumination the frequency of channel opening fluctuated considerably over time, and there were occasional periods (see Fig. 1, bottom trace) in which the frequency of opening was substantially reduced relative to the dark level but was not abolished. In nine experiments, channel properties during such periods of reduced channel activity were compared with properties in the dark before and after the steady illumination. The results are presented in Table 2. Burst duration, which is a measure of the over-all duration of each channel event ignoring brief closures, was consistently briefer during illumination than in the dark. The average open duration, which is a measure of the average duration of each excursion into the open state regardless of whether it occurs in isolation or as part of a burst, was also shortened by non-saturating illumination. The average closed duration within a burst was not affected by light. Thus, non-saturating light reduced the probability that the channel would enter the open state not only by reducing the over-all rate of channel events, but also by reducing the average duration of each event (burst duration) and of the excursions into the open state within an event.

External perfusion with 0 Ca^{2+} solution induces light-sensitive channel activity in silent patches

Approximately one-half of all cell-attached patches did not exhibit light-suppressable channel activity, even in rods that had been shown by suction-electrode recordings to possess normal dark current. To determine if such silent patches lack light-sensitive channels, we superfused the outer segment with low-Ca^{2+} solution. Removal of external Ca^{2+} has been shown to increase the dark current dramatically (Hodgkin *et al.* 1984) and thus might be expected to open light-sensitive channels in

silent patches if they were present. As shown in Fig. 7, large amounts of light-sensitive channel activity could be induced in this manner. The chart-recorder traces in Fig. 7*A* show a low-resolution view of the over-all experiment, and the high-resolution traces in Fig. 7*B* show light-sensitive channel activity. With a delay, 0 Ca^{2+} perfusion induced channel events that could be eliminated by light and that appeared

TABLE 2. Effect of non-saturating illumination on the light-sensitive channel. Except for the last column, numbers are ratios giving for each parameter the value during illumination divided by the average of the values in darkness before and after the light. Abbreviations: t_b, average burst duration; t_o, average open duration; t_c, average closed duration within a burst; f, frequency of channel events; p(open), the proportion of total time that the channel was in the open state; I, light intensity in photons μm^{-2} s^{-1}

Experiment number	t_b	t_0	t_c	f	p(open)	I (photons μm^{-2} s^{-1})
1	0·54	0·71	1·04	0·42	0·25	2·3
2	0·67	0·91	1·21	0·16	0·09	3·8
3	0·76	0·79	0·74	0·24	0·08	2·3
4	0·80	0·90	0·96	0·19	0·21	0·6
5	0·54	0·74	0·89	0·33	0·18	2·2
6	0·72	0·90	1·00	0·14	0·08	1·1
7	0·75	0·93	0·85	0·43	0·25	0·6
8	0·59	0·80	0·94	0·28	0·17	3·5
9	0·64	0·83	1·27	0·24	0·20	0·9
Mean	0·67*	0·83*	0·99	0·27*	0·16*	
S.E.	0·03	0·03	0·06	0·04	0·02	

* $P < 0·01$ that true ratio was 1·0; 99% confidence interval estimated using t statistic (Hays, 1963).

to be indistinguishable from light-sensitive channel events in other patches. Channel openings became more frequent with prolonged perfusion and fused into a large, noisy inward current. Upon removal of the 0 Ca^{2+} perfusion, all channel activity vanished. Similar results were observed in all eight experiments of this type. A minimum of about thirty channels would be required to produce the maximum mean current during 0 Ca^{2+} perfusion in Fig. 7. This estimate takes into account the fact that channel events occur as bursts of brief openings and closings, so that the average current during an event is less than the peak channel current during an opening. The total number of channels in the patch was likely to be greater than thirty because the probability of channel opening was likely to be less than 1·0.

Because the external face of the recorded patch was already exposed to 0 Ca^{2+}, 0 Mg^{2+} Ringer solution and was separated from the rest of the external bath by a gigaohm seal, the effect of 0 Ca^{2+} perfusion on channel activity must represent an internal action. As demonstrated previously (Matthews, 1985*d*, 1986*c*), 0 Ca^{2+} solution applied directly to the intracellular face of excised patches has no specific effect on patch conductance. Therefore, the action of external 0 Ca^{2+} solution on light-sensitive channel activity reflects an indirect internal effect, possibly on cyclic GMP metabolism.

The onset of channel activity following 0 Ca^{2+} perfusion was slow compared with previously reported effects of low external Ca^{2+} on the membrane current of the

entire outer segment (Hodgkin *et al.* 1984; Matthews, 1985*e*). In Fig. 7, there was a delay of approximately 20 s between onset of superfusion and the beginning of a large increase in channel activity, whereas Hodgkin *et al.* (1984) showed that macroscopic dark current begins to increase rapidly after a reduction in external Ca^{2+}

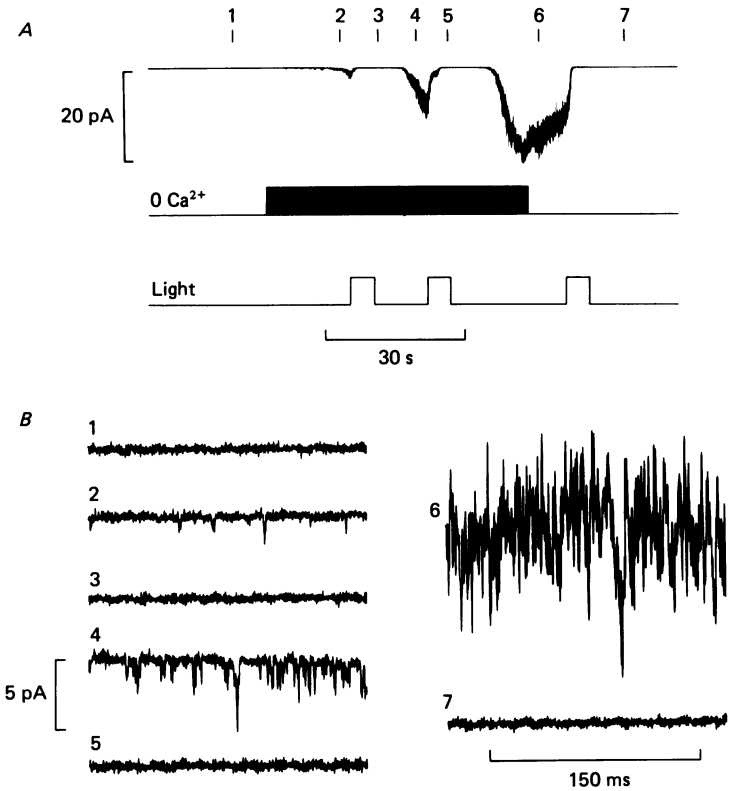

Fig. 7. Perfusion of the outer segment with 0 Ca^{2+} Ringer solution induced light-sensitive channel activity in a cell-attached patch that initially lacked channel activity. *A*, chart recorder traces at low gain. The top trace shows patch membrane current, the middle trace shows timing of 0 Ca^{2+} perfusion, and the bottom trace shows timing of light stimuli. Light intensity was 160 photons μm^{-2} s^{-1}. 0 Ca^{2+} Ringer solution was the same as normal Ringer solution, except that no $CaCl_2$ was added and 0·3 mM-EGTA was included. Patch hyperpolarized by 82 mV. *B*, higher-resolution views of light-sensitive channel activity. Timing of individual samples during the experiment are indicated by the corresponding numbers above the chart recorder trace in *A*. Band width = 0–2000 Hz. Before (trace 1) and after (trace 7) 0 Ca^{2+} perfusion there was no channel activity in darkness. With a delay after onset of perfusion, inward-current channel events appeared (trace 2) that could be abolished by illumination (traces 3 and 5). With time, channel events became more frequent (trace 4) and finally fused into a large noisy inward current (trace 6) that appeared to represent the superimposed openings of a large number of light-sensitive channels.

concentration and reaches a peak in about 10 s. The reason for this difference is unknown, but there are several possible explanations. One contributing factor may have been differences in the speed of solution change; Hodgkin *et al.* used a rapid perfusion system, while 0 Ca^{2+} solution was applied in the present experiments by

manually lowering a perfusion pipette to the recorded cell. The speed of solution change was not measured, but the positioning of the pipette was accomplished within 1–2 s. Another possibility is that the response of the macroscopic dark current reflects both external and internal actions of removing Ca^{2+}, while the patch response reflects only internal actions, the external face within the patch pipette already being exposed to low Ca^{2+} solution. It is also possible that the channels in silent patches are in a different state from active channels, and that removal of Ca^{2+} converts them to the active configuration with a slow time course.

In patches that showed light-sensitive channel activity under normal recording conditions, external perfusion with 0 Ca^{2+} solution increased the frequency of channel events. This action was routinely observed because of the standard procedure of perfusing the rod with 0 Ca^{2+}, 0 Mg^{2+} Ringer solution before attempting to excise an inside-out patch (to minimize chances of vesicle formation; see Methods). Perfusion with 0 Ca^{2+}, 0 Mg^{2+} Ringer solution always caused a large increase in light-sensitive channel opening in responsive patches. Upon removal of the perfusion, the rate of channel opening returned to its low, basal level. This demonstrates that there was a large number of individual channels in each patch; the lower limit was typically 20–70 channels, based on the maximal mean current achieved during 0 Ca^{2+}, 0 Mg^{2+} perfusion and the estimated single-channel current. Because this lower limit would require that all channels are open all the time, the actual number of channels per patch is likely to be higher.

The area of the recorded membrane patch was uncertain because the amount of membrane entering the pipette during seal formation was unknown. In these experiments, however, there was no visible entry of membrane into the pipette, and there was little or no visible constriction of the rod outer segment at the point of contact with the patch pipette. Both of these observations suggest that the amount of membrane entering the pipette was small and thus that the area of the orifice at the tip of the pipette can be used as a rough approximation of the patch area. The inner diameter of the tip was typically about 0·5 μm, giving a lower limit of about 0·2 μm^2 for patch area. Thus, from the lower limit of 20–70 channels per patch and the lower limit of 0·2 μm^2 for patch area, the estimated channel density is 100–350 channels μm^{-2}.

Comparison of light-sensitive channel with cyclic GMP-activated channel

In this section, we consider the properties of single cyclic GMP-activated channels in excised patches of outer segment membrane and compare that channel with the light-sensitive channel of intact rods discussed in the first section. The cyclic GMP-sensitive conductance was activated by applying cyclic GMP to the intracellular face of an inside-out patch (Fesenko *et al.* 1985; Nakatani & Yau, 1985). In a number of experiments, it proved possible to excise a patch in inside-out configuration after recording light-sensitive channel activity in the same patch while it was still attached to the rod. Those experiments allowed a direct comparison of light-sensitive and cyclic GMP-activated channels in the same membrane (Matthews, 1987). Samples of channel activity from one such experiment are shown in Fig. 8. In cell-attached recording from the intact rod, light-suppressable channel events were observed (left panel), as described earlier. After excision, application of 10 μM-cyclic GMP in

0 Ca^{2+}, 0 Mg^{2+} Ringer solution to the intracellular face opened a channel that appeared indistinguishable from the light-sensitive channel observed previously in the intact rod.

As an over-all comparison of the two types of channel events, the power spectral densities of the light-sensitive and cyclic GMP-activated channel activity were compared. The difference spectrum of the light-sensitive channel was obtained by subtracting the power spectrum in bright light, which suppressed all channel activity, from the power spectrum in darkness. The difference spectrum of the cyclic GMP-activated channel was obtained by subtracting the power spectrum during perfusion of the inner face of the membrane with 0 Ca^{2+}, 0 Mg^{2+} Ringer solution from the spectrum during perfusion with 0 Ca^{2+}, 0 Mg^{2+} Ringer solution + 10 μM-cyclic GMP. As shown by Matthews (1987) the two difference spectra were superimposable, suggesting that the two channels had the same over-all kinetics. Both spectra could be described by a sum of two Lorentzian components, with corner frequencies of 75 ± 8 and 1080 ± 120 Hz for the light-sensitive channel and 85 ± 5 and 1000 ± 130 Hz for the cyclic GMP-activated channel. To compare results across experiments, the low-frequency and high-frequency corner frequencies from the power spectrum of the cyclic GMP-activated channel were divided by the corresponding corner frequencies from spectra of the light-sensitive channel in the same patch. If the power spectra of the two types of channel activity were the same, these ratios would be 1·0. In seven experiments, the average ratio was $0{\cdot}93 \pm 0{\cdot}083$ (mean ± S.E.) for the low-frequency corner frequency and $1{\cdot}11 \pm 0{\cdot}17$ for the high-frequency corner frequency. Neither ratio is significantly different from 1·0. Thus, comparison of power spectra confirmed the visual impression that the light-sensitive and cyclic GMP-activated channel events within the same patch had the same form.

Comparison of temporal parameters of light-sensitive and cyclic GMP-activated channel

The duration of a burst of opening of the cyclic GMP-activated channel was measured in the same way as the light-sensitive channel, i.e. closures less than 1 ms in duration were ignored. An example of the distribution of burst durations for cyclic GMP-activated channels in an excised patch is shown in Fig. 3 (triangles). Superimposed on the same graph is the distribution of burst durations for light-sensitive channels in the same patch of membrane before excision (circles). Like the light-sensitive channel, the distribution for the cyclic GMP-activated channel was well described by a single exponential, except for the briefest bin, at durations up to 4 ms. The straight line through the data corresponds to an exponential time constant of 0·94 ms. The distribution of brief burst durations is shown on expanded time scale in the inset. Again, distributions from light-sensitive and cyclic GMP-activated channels were similar. Both were fitted by a single exponential distribution with time constant of 0·1 ms.

The distribution of open durations for cyclic GMP-activated channel activity was obtained as described earlier for the light-sensitive channel (i.e. an individual opening was terminated by any closure, no matter how brief). The distribution of open duration for cyclic GMP-activated and light-sensitive channels in the same patch are shown in Fig. 4*A*. Both distributions were fitted by an exponential distribution with

time constant of 0·17 ms. The distribution of closed duration within a burst (omitting long closures between individual bursts) from the same experiment is shown in Fig. 4*B*. Again, distributions from light-sensitive and cyclic GMP-activated channel activity were similar and could be fitted by a single exponential distribution with time constant 0·33 ms.

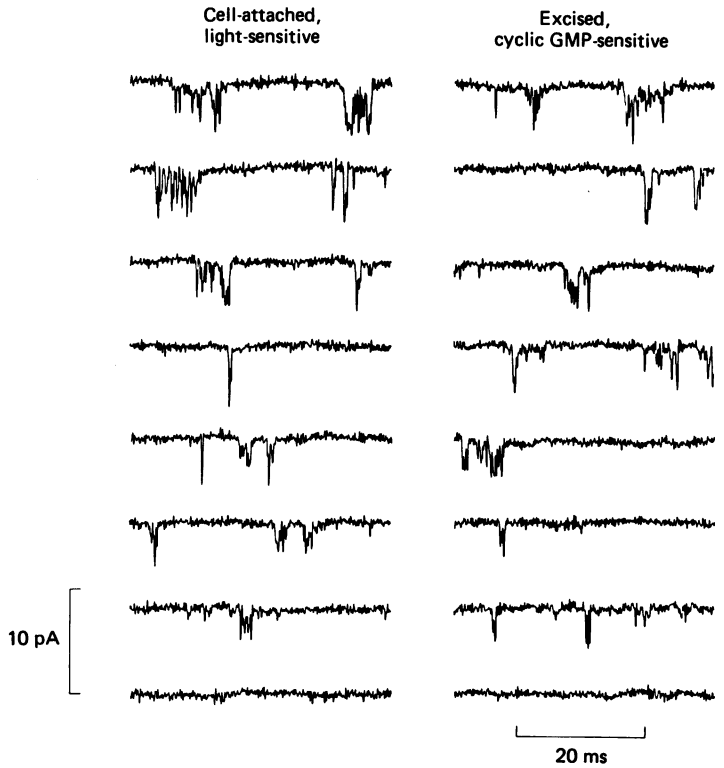

Fig. 8. Comparison of light-sensitive and cyclic GMP-sensitive channel activity within the same patch of outer segment membrane. Traces in the left column show light-sensitive channel activity in a cell-attached recording from an intact rod. The bottom trace is during saturating light, and all others are in darkness. The patch was hyperpolarized by 148 mV. Traces in the right column show cyclic GMP-sensitive channel activity in the same patch of membrane after detaching the patch from the rod. In the bottom trace, the intracellular face of the patch was perfused with 0 Ca^{2+}, 0 Mg^{2+} Ringer solution. In all other traces, the intracellular face was perfused with 0 Ca^{2+}, 0 Mg^{2+} Ringer solution + 10 μM-cyclic GMP. The patch electrode was filled with 0 Ca^{2+}, 0 Mg^{2+} Ringer solution. The patch membrane potential was −148 mV. Band width = 0–4000 Hz.

The average burst duration, open duration, and closed duration within a burst for experiments on the cyclic GMP-activated channel are summarized in Table 1. In eleven experiments, burst duration averaged 0·85 ± 0·07 ms (mean ± S.E.), which is similar to the burst duration of 0·78 ± 0·03 ms in experiments on the light-sensitive channel. In the seven experiments in which comparison could be made within the same patch, the ratio of cyclic GMP-activated to light-sensitive burst duration averaged 1·09 ± 0·08 (mean ± S.E.), which is not significantly different from 1·0. As shown in Table 1, the cyclic GMP-activated channel was also similar to the light-

sensitive channel in average open duration and average closed duration within a burst. Thus, the cyclic GMP-activated channel was indistinguishable from the light-sensitive channel on the basis of temporal parameters of channel opening and closing.

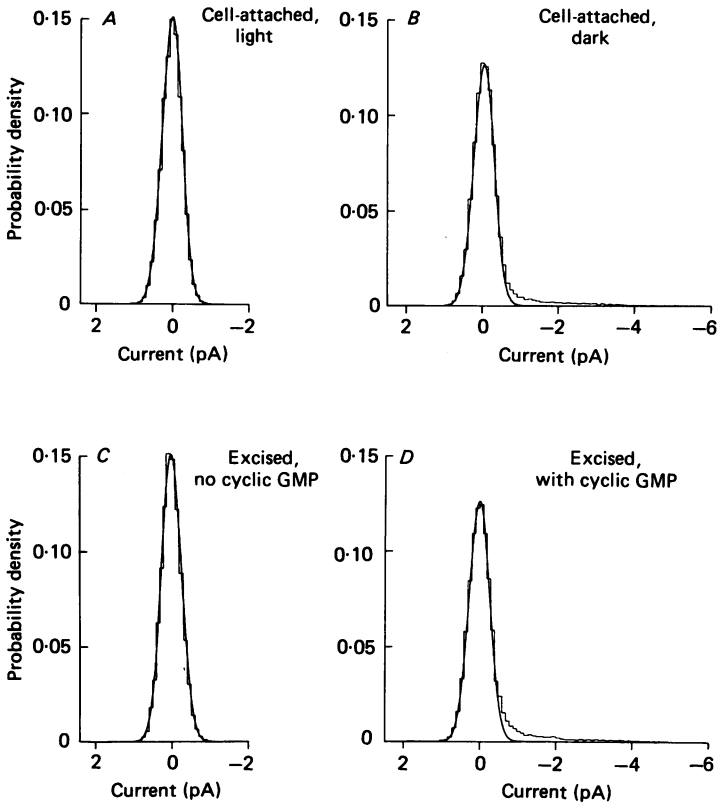

Fig. 9. Probability density functions for amplitude of patch membrane current. Bin width = 0·2 pA; band width = 0–4000 Hz. *A*, cell-attached recording during bright light. The patch was hyperpolarized by 148 mV. The smooth curve was drawn according to a Gaussian distribution with a standard deviation of 0·27 pA. *B*, cell-attached recording in darkness. The smooth curve has the same standard deviation as in *A*, but the height was adjusted to fit the experimental density function. *C*, recording from excised patch without cyclic GMP on the intracellular face of the membrane. Patch membrane potential = −148 mV. Same patch as in *A* and *B* after detachment from the rod in inside-out configuration. The smooth curve was drawn according to a Gaussian distribution with standard deviation of 0·27 pA, the same as for the cell-attached patch in bright light. *D*, excised patch with 10 μM-cyclic GMP on intracellular face of patch. The smooth curve was drawn as in *B*.

Probability density functions of current amplitude

As a further comparison of the cyclic GMP-activated and light-sensitive channels, probability density functions of current amplitude were obtained for light-sensitive and cyclic GMP-activated channel activity in the same membrane patch. Results from one experiment are shown in Fig. 9. During saturating illumination, the probability density function of membrane current recorded from the intact rod was

Gaussian, with a standard deviation of 0·27 pA (Fig. 9*A*). Because all channel activity was abolished by the light, this gives the probability density function of base-line fluctuations. In darkness the probability density function consisted of a central Gaussian corresponding to base-line fluctuations and a negative tail corresponding to inward current flowing when channels open (Fig. 9*B*). The central Gaussian had the same standard deviation as in saturating light.

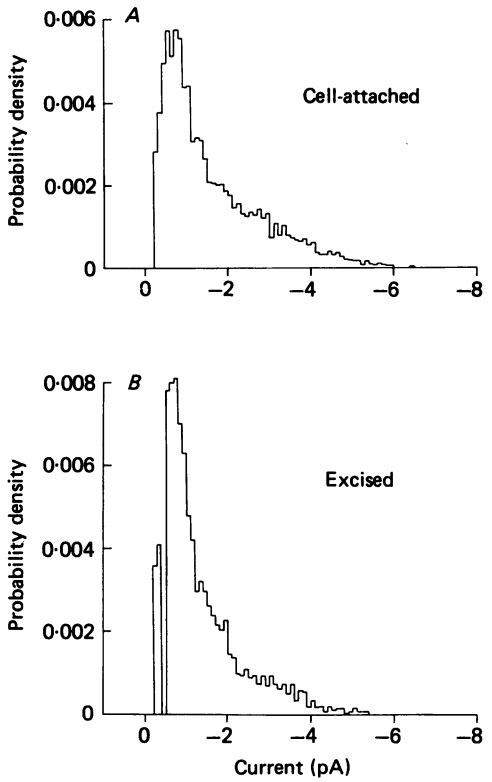

Fig. 10. Higher resolution views of probability density of current amplitude after subtracting out Gaussian probability density function with standard deviation of 0·27 pA. Bin width = 0·2 pA; band width = 0–4000 Hz. Only the negative tail corresponding to inward current through open channels is shown. *A*, light-sensitive channel activity in cell-attached recording from intact rod. *B*, cyclic GMP-activated channel activity in the same patch after detachment.

After excising the patch, the probability density function in the absence of cyclic GMP was again Gaussian, with a standard deviation of 0·27 pA (Fig. 9*C*). Thus, the base-line fluctuations in the membrane current of the excised patch were similar to those of the attached patch in saturating light. This suggests that the base-line noise in the intact cell originates within the recorded patch and the apparatus and includes no component attributable to intracellular physiological events. Upon addition of cyclic GMP to the perfusate flowing past the intracellular face of the membrane, a negative tail like that in darkness in cell-attached recordings appeared (Fig. 9*D*).

This represents inward current (at positive pipette holding potentials) through cyclic GMP-activated channels.

The component due to current through open channels can be seen more clearly in Fig. 10, which shows probability density functions for light-sensitive (*A*) and cyclic GMP-activated (*B*) channel activity after subtracting out the Gaussian fitted to the central peak. The two distributions of current amplitude were similar.

Channel conductance

It is apparent from inspection of Fig. 10 that the amplitude of the current due to channel activity was approximately the same before and after excising the patch. The holding potential inside the patch pipette relative to bath ground was the same for both recordings; however, the driving force for current in the excised patch was significantly less than in the intact cell, because of the dark driving force of approximately 50 mV (see above) added to the pipette potential in the latter instance. This suggests that the channel conductance was larger in the excised patch than in the intact cell. To examine this more directly, we estimated single-channel conductance by determining the relation between single-channel current and patch membrane potential. Fig. 11 shows results of one such experiment on light-sensitive and cyclic GMP-activated channels in the same patch. In this experiment, the conductance of the cyclic GMP-activated channel in the excised patch was estimated to be 22·4 pS, while the light-sensitive channel of the intact cell had an estimated single-channel conductance of 17·4 pS. Results from all experiments on excised patches (Table 1) show that on average the conductance of the cyclic GMP-activated channel was slightly higher than that of the light-sensitive channel (24·0 *vs.* 20·5 pS), although the difference is of marginal statistical significance (two-tailed t test; $t = 2{\cdot}163$, $P < 0{\cdot}05$).

In the intact rod, the intracellular face of the membrane was bathed in intracellular fluid, whereas in excised patches the intracellular fluid was replaced by 0 Ca^{2+}, 0 Mg^{2+} Ringer solution. Differences in the ionic compositions of the two fluids might account for differing channel conductance in intact cells and excised patches. For example, the total concentration of divalent cations was likely lower in the 0 Ca^{2+}, 0 Mg^{2+} Ringer solution than in intracellular fluid, and this might contribute to a higher channel conductance in excised membranes. Another difference between intracellular fluid and 0 Ca^{2+}, 0 Mg^{2+} Ringer solution would likely be the concentrations of sodium and potassium ions. However, under the experimental conditions, the effect of variation in the concentrations of permeant ions bathing the intracellular membrane face on apparent channel conductance would likely have been minimized because most or all of the measurements were made with strong driving force for inward membrane current (see Figs. 6 and 11). Under these conditions, the concentrations of sodium and potassium ions within the pipette would be expected to be more important than intracellular concentrations.

Effect of patch membrane potential on temporal parameters

In experiments on the light-sensitive channel, we demonstrated that hyperpolarization of the recorded patch had little effect on the average burst duration

or on the average open duration. For comparison, we also examined the effect of membrane potential on these temporal parameters of the cyclic GMP-activated channel of excised patches. Results from five experiments are summarized in Fig. 5*C* and *D*. In order to compare results across experiments, durations are expressed relative to the duration at 0 mV obtained by extrapolation of a straight line fitted

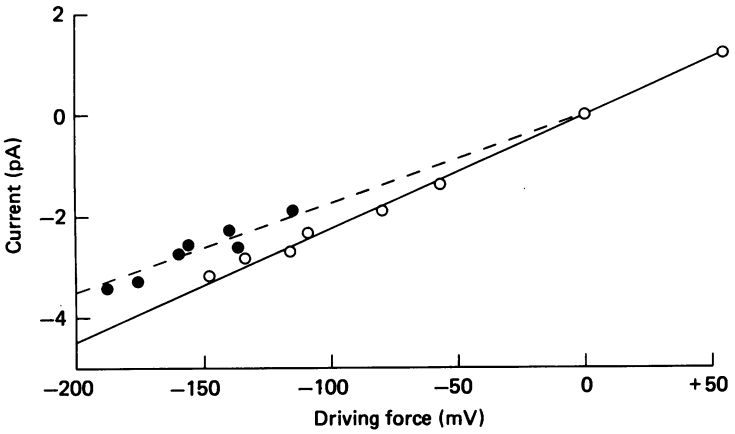

Fig. 11. Relation between average amplitude of single-channel current and voltage driving force for light-sensitive (filled circles) and cyclic GMP-activated channels (open circles) of the same patch. The driving force was varied by clamping the pipette potential at different levels. For the light-sensitive channel, driving force was estimated by extrapolating the straight line fitted to the data to zero current. For the cyclic GMP-activated channel in the excised patch, driving force was equal to the holding potential because the reversal potential was 0 mV in the symmetrical ionic solutions bathing the two sides of the membrane.

to the data from each experiment. As with the light-sensitive channel, increasingly negative membrane potential had little effect on the average open duration of the cyclic GMP-activated channel (Fig. 5*C*). Straight lines fitted to individual experiments had slopes corresponding to an increase in duration of $1{\cdot}4 \pm 0{\cdot}2$ μs per millivolt of hyperpolarization (mean $\pm$ S.E.). The comparable value was 0·4 μs per millivolt for the average open duration of the light-sensitive channel. The average burst duration of the cyclic GMP-activated channel (Fig. 5*D*) was also virtually unaffected by hyperpolarization, with an average increase of $0{\cdot}7 \pm 0{\cdot}6$ μs per millivolt of hyperpolarization. Again, this was similar to the average increase of 0·8 μs per millivolt observed for the burst duration of the light-sensitive channel. Thus, the temporal properties of both the light-sensitive and the cyclic GMP-sensitive channel were little affected by hyperpolarization.

DISCUSSION

A principal result reported here is the resolution of single openings of the light-sensitive channel in functioning, dark-adapted rod photoreceptors. The size of the single-channel conductance in the absence of external divalent cations (20·5 pS, on

average) establishes that the channel is a pore, not a carrier. The small size of inward current through a single channel under physiological conditions (Bodoia & Detwiler, 1984*b*) apparently results from the blocking action of external divalent cations. When divalent cations were removed from the external face of the membrane, hyperpolarization of the patch readily increased inward current through the light-sensitive channel in a linear manner. This behaviour suggests that the usual strong outward rectification of the light-sensitive conductance (Bader, MacLeish & Schwartz, 1979; Baylor & Nunn, 1986) is lost when divalent cations are removed from the extracellular medium. In this regard, the light-sensitive channel appears to behave like the cyclic GMP-activated conductance, in which the direction of rectification depends on the direction of the divalent cation gradient across the membrane (Matthews, 1986*a*). The effect of divalent cations on rectification is similar to that reported previously for glutamate-activated channels (Dekin, 1983; Nowak, Bregestovski, Ascher, Herbet & Prochiantz, 1984), where external Mg^{2+} causes flickery block of the channel and outward rectification of the current–voltage relation.

Effect of low external Ca^{2+} on frequency of channel events

Although one effect of extracellular Ca^{2+} is on single-channel current, external Ca^{2+} must have an intracellular action, as well. Superfusion of the outer segment with low-Ca^{2+} solution increased the frequency of opening of light-sensitive channels in a cell-attached patch. Because the external face of the recorded patch was inaccessible within the patch pipette and was already exposed to the low-Ca^{2+} solution used to fill the pipette, this effect of removing external Ca^{2+} must have been an intracellular action. The nature of this internal action is unknown at present.

Superfusion with low-Ca^{2+} solution also induced light-sensitive channel activity in silent patches that showed no channel activity under normal conditions. This demonstrates that light-sensitive channels are present in silent patches. It is puzzling, however, why these channels seem never to open under normal circumstances. Some possible explanations include: regional variation in concentration of internal transmitter, such as might arise with some form of compartmentalization; an inactive form of the channel, which can be reversibly transformed in some way into the active form when Ca^{2+} concentration falls; and chronic activation of the phototransduction machinery in part of the rod, locally shutting all light-sensitive channels.

Channel flicker

Both light-sensitive and cyclic GMP-activated channels showed pronounced flicker in the open state (e.g. Figs. 2 and 8). This flicker might be due to blockade of the channel by residual small amounts of Ca^{2+} and Mg^{2+} within the patch pipette. It is likely, for example, that some amount of normal Ringer solution from the bathing solution entered the patch pipette when suction was applied to form a gigaohm seal, thus raising the concentration of divalent cations within the pipette. Haynes *et al.* (1986) have shown that Ca^{2+} and Mg^{2+} produce a rapid, concentration-dependent flickery block of the cyclic GMP-activated channel of excised patches. An alternative, however, is that the flicker represents some gating transition intrinsic to the channel. For example, flicker might be caused by spontaneous transitions between conducting

states of different size, rather than by complete closure of the channel. Resolution of this question will require further experiments.

The results of earlier experiments employing noise analysis (Matthews, 1986*a*, *c*) suggested that the cyclic GMP-activated channel is not a two-state open-or-closed channel. The evidence was that the power spectrum of cyclic GMP-activated current noise was fitted by the sum of two Lorentzian components, like acetylcholine-activated channel noise at the neuromuscular junction in the presence of blocking drugs (Colquhoun & Sheridan, 1981), and that the relation between mean and variance of cyclic GMP-sensitive current was linear all the way to saturation, rather than parabolic as expected of a two-state channel. One explanation of these observations was that the channel flickered rapidly in the open state, an explanation since confirmed by single-channel recordings (Haynes *et al.* 1986; Zimmerman & Baylor, 1986; present experiments).

Effect of voltage on the light-sensitive channel

In intact rods, hyperpolarizing voltage steps have been reported to cause a time-dependent increase in the dark current (Baylor & Nunn, 1986). In the present experiments, there was little indication that the light-sensitive channel was voltage sensitive. Even large hyperpolarization left the average open duration and the average burst duration effectively unchanged. There was a small increase in the frequency of events when the patch was hyperpolarized, but the magnitude of this effect seems insufficient to explain the increase in dark current observed by Baylor & Nunn (1986). This failure of the light-sensitive channel to respond to hyperpolarization of the recorded patch lends support to Baylor and Nunn's suggestion that the effect of voltage on the light-sensitive current is indirect, perhaps acting via the electrogenic Na–Ca exchanger of the rod plasma membrane (Yau & Nakatani, 1984). Their notion is that hyperpolarization would speed the exchanger and lower internal Ca^{2+} concentration, which would in turn increase the light-sensitive current. In the present experiments, hyperpolarization was applied to a small portion of the total plasma membrane, and the over-all rate of Na–Ca exchange in the entire outer segment would be expected to be little affected. Perhaps the small and variable increase in frequency of channel events observed here reflects the small increase in over-all membrane potential of the cell caused by hyperpolarizing the recorded patch; such an increase in membrane potential might then increase frequency of channel opening via an indirect mechanism of the type suggested by Baylor & Nunn (1986).

Comparison of light-sensitive and cyclic GMP-activated channels in the same patch

Another principal result of the present experiments was the comparison of light-sensitive and cyclic GMP-activated channels within the same patch of outer segment plasma membrane. The light-sensitive channel recorded in a patch attached to an intact, dark-adapted rod was essentially indistinguishable from the cyclic GMP-activated channel recorded later from the same patch after detachment from the rod. These experiments leave little doubt that the channel closed by light in the intact rod is the same as the channel opened by cyclic GMP in the excised patch. There is a wealth of biochemical evidence demonstrating light-stimulated hydrolysis of cyclic

GMP in rods, and the details of the machinery linking photoisomerization of rhodopsin to reduction in cyclic GMP concentration are now well known (see Stryer, 1986, for a recent review). Coupled with the present results demonstrating that the light-sensitive and the cyclic GMP-activated channel are identical, the clear implication is that cyclic GMP is the internal transmitter responsible for keeping the light-sensitive channel open in darkness, and that light-stimulated hydrolysis of cyclic GMP causes the channel to close upon illumination.

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