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MEMBRANE CURRENT NOISE IN TOAD RETINAL RODS EXPOSED TO LOW EXTERNAL CALCIUM

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SUMMARY

1. Outer segment membrane current of single rod photoreceptors from toad retina was recorded with a suction electrode, and extracellular calcium concentration was manipulated by transferring the recorded cell from one pool of saline to another or by locally perfusing the outer segment.

2. The large increase in dark current that resulted from exposure to low-calcium saline was accompanied by an increase in dark noise in the band 1–800 Hz. This noise was suppressed by bright light, and its power spectrum could be described by a single Lorentzian equation with average corner frequency of $40{\cdot}1 \pm 9{\cdot}5$ Hz (mean $\pm$ S.D., $n = 11$).

3. In low-calcium saline, saturating flash responses were often followed by a transient increase in the dark current lasting 30–100 s. During this rebound period of increased dark current, increased dark noise similar to that described in 2 was observed. The power spectrum of this noise was also fitted by a single Lorentzian equation, with corner frequency averaging $29{\cdot}7 \pm 6{\cdot}6$ Hz (mean $\pm$ S.D., $n = 27$).

4. To examine the possible role of intracellular voltage fluctuations in generating the noise, suction electrodes were filled with calcium-free saline and recordings were made from outer segments of rods attached to pieces of retina. In this recording configuration, the electrical coupling among the rods in the piece should attenuate voltage fluctuations associated with the post-light rebound period of increased dark current. In this situation, the rebound increase in dark current was still observed, but the noise was reduced or absent. Using the same recording configuration, isolated rods showed pronounced noise during the rebound.

5. The result in 4 suggests that the noise resulted from fluctuations in intracellular voltage, not directly from fluctuations in the light-sensitive channels. In this view, the corner frequency of the noise power spectrum probably reflects the membrane time constant of the isolated rod.

INTRODUCTION

Reducing external calcium concentration is known to increase the dark current of rod photoreceptors (Bastian & Fain, 1972; Hagins & Yoshikami, 1974; Yau, McNaughton & Hodgkin, 1981; Hodgkin, McNaughton, Nunn & Yau, 1984). The

experiments reported here examine membrane current noise accompanying the increase in dark current in low calcium. The rationale was that low external calcium might reveal noise originating in the light-sensitive channels of the outer segment either by increasing the channel conductance or by increasing the number of open channels and hence the total variance. When the outer segment of an isolated toad rod was exposed to saline containing no added calcium and 2 mM-EGTA, there was a pronounced increase in membrane current noise. This noise could be suppressed by bright light, and its power spectral density could be fitted by a single Lorentzian equation. However, indirect experiments suggested that the noise arose from fluctuations in intracellular voltage, rather than directly from the light-sensitive conductance mechanism of the outer segment. A preliminary report of some of these results has appeared (Matthews, 1984).

METHODS

Preparation and recording

Suction-electrode recording of membrane current and preparation of toad (*Bufo marinus*) retinas were carried out as described by Baylor, Lamb & Yau (1979) and Baylor, Matthews & Yau (1980). In most experiments, recordings were made from isolated rods that were detached from small pieces of chopped retina (Yau *et al.* 1981; Hodgkin *et al.* 1984). These cells typically appeared to be intact except for the synaptic terminal and gave light responses similar to those of cells in pieces for up to several hours. In some experiments, the inner segment was drawn into the suction electrode and the outer segment was exposed to the external fluid outside the electrode. In other experiments, recordings were made from the outer segment, leaving the inner segment outside the suction electrode. Responses are plotted according to the usual convention that an outward current is positive. Thus, light responses appear as upward deflexions with the outer segment within the suction electrode and downward deflexions with the inner segment inside the electrode. The terms light-sensitive conductance and light-sensitive channels will be used to denote the membrane mechanism carrying the light-sensitive current in the outer segment. These terms are not meant to restrict the range of possible mechanisms.

Solution changes

The external bathing solution was altered either by locally perfusing the region around the tip of the suction electrode or by transferring the recorded cell and the suction electrode from one pool of saline to a new pool containing saline of the desired composition. The transfer procedure is illustrated diagrammatically in Fig. 1. In order to protect the cell during the transfer through the air–water interfaces of the two pools, the cell was drawn entirely inside the electrode and then returned to recording position (i.e. with the outer segment protruding) after the electrode was in place in the new pool of saline. In the present experiments, the chamber was constructed with five independent pools, thus allowing up to five solutions to be tested on each cell. In other experiments employing local perfusion, the outer segment was drawn into the electrode. The inner segment was then locally perfused with altered saline via a separate perfusion pipette positioned near the recorded cell (Liebman, Mueller & Pugh, 1984).

The composition of the saline was: NaCl, 111 mM; KCl, 2·5 mM; $MgCl_2$, 1·6 mM; EDTA, 20 μM; glucose, 10 mM; HEPES, 10 mM; pH = 7·8 with NaOH. In normal saline, calium concentration was 1·0 mM; calcium-free saline was made with no added calcium and 2 mM-EGTA. The pH of calcium-free saline was readjusted to 7·8 after addition of EGTA.

In experiments with suction electrodes, uncertainty concerning the extent of solution mixing between the interior of the suction pipette and the external fluid in the chamber complicates interpretation of effects of solution changes. In the present experiments, the seal between the suction pipette and the rod membrane was similar to that reported by others (e.g. Baylor *et al.* 1979; Hodgkin *et al.* 1984): upon drawing the rod into the electrode, the resistance of the pipette increased 3–10-fold to a final resistance of 5–12 MΩ. Under similar conditions, Hodgkin *et al.* (1984) reported that some mixing of solutions within the tip of the pipette could occur. Thus, it is possible

that local perfusion of the portion of the rod outside the electrode might affect the portion inside the electrode.

Data acquisition and analysis

Membrane current was recorded via a current-to-voltage transducer connected to the interior of the suction pipette. This signal, together with synchronizing pulses and light monitor output, was recorded on a FM tape recorder at band width 0–312 Hz. Selected segments were digitized and

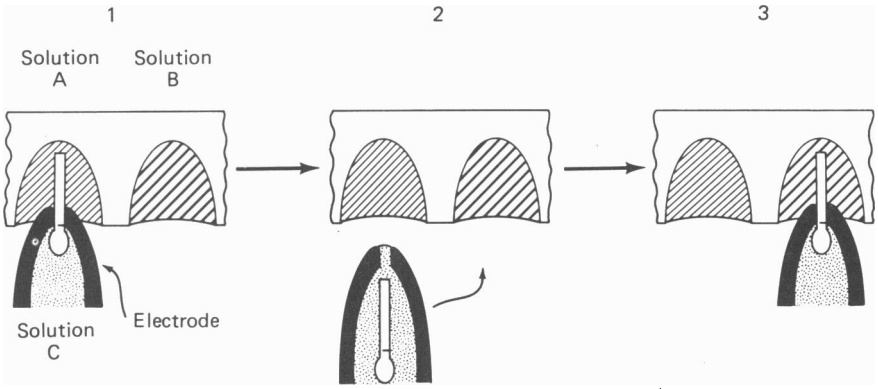

Fig. 1. Schematic representation of procedure for transferring cells from one type of bathing fluid to another. Not drawn to scale. Volume of each pool of saline was actually 0·6 ml. Only two of the five available pools in the chamber are shown in the diagram, which gives a top view. Each pool was open only in the front (toward electrode). The top and bottom of the chamber were silanized microscope slides, the sides were Plexiglas, and the fluid remained in each pool by surface tension. The inner segment of an isolated rod was drawn into a suction electrode for recording membrane current (1). To change the fluid bathing the outer segment, the cell was drawn completely into the electrode. The electrode and cell were then removed from the solution and moved into position to enter the new solution (2). After the electrode was placed into the new pool, the cell was returned to recording position with the outer segment exposed to the new solution (3). All manipulations were made under infra-red illumination while viewing the preparation through an inverted, compound microscope equipped with an infra-red-sensitive television system.

analysed in a computer (Chrislin Industries, CI-11/23-AC). For power spectral analysis, data were usually digitized in sweeps consisting of 512 or 1024 points at 2 ms per point after passing through a variable bandpass filter. The low-frequency cut-off was usually set to 1 Hz and the high-frequency cut-off to 150 Hz, which was the approximate frequency at which the signal power fell to the level of the thermal noise determined by the leakage resistance at the tip of the suction pipette. Before calculation of the power spectrum for each sweep, any linear trend was removed digitally and the first and last 10 % of the data points were cosine-tapered (Bendat & Piersol, 1971).

Light stimuli

Illumination was controlled by a dual-beam optical stimulator similar in design to that of Baylor & Hodgkin (1973). One beam provided infra-red light ($\lambda > 850$ nm) for viewing manipulations, and the other gave visible light (500 nm, set by an interference filter with half band width 10 nm) for stimulating the rods. Light intensity was controlled by calibrated neutral density filters. At the end of each experiment, light intensity was measured by placing the detector of a radiometer (UDT Model 111A) on the stage of the microscope in the position normally occupied by the preparation.

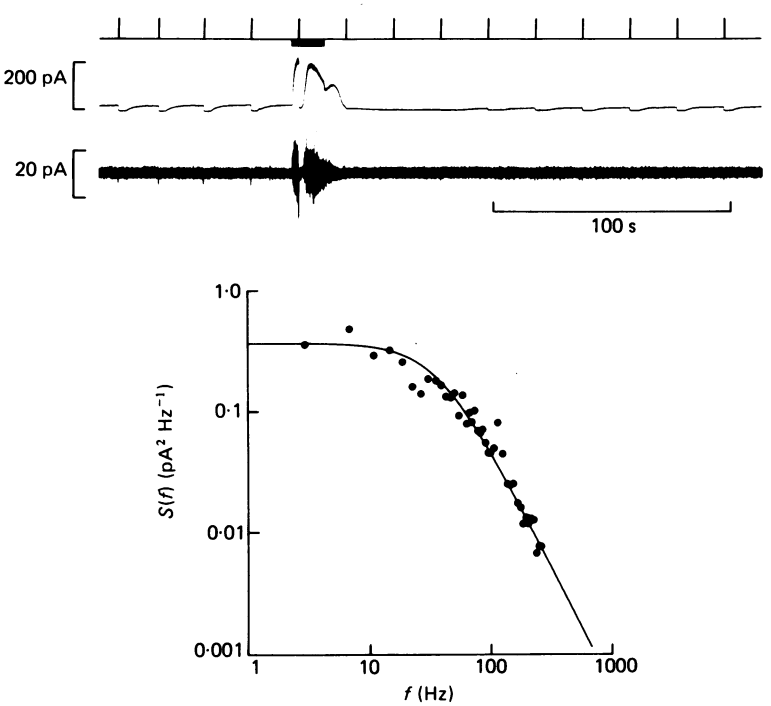

Fig. 2. Effect of brief perfusion of a rod outer segment with saline containing no added calcium and 2 mM-EGTA (calcium-free). Middle trace above shows a low-gain chart recorder record of membrane current (band width: 0–100 Hz), and the lower trace shows a higher-gain, a.c.-coupled record of the same data (band width: 10–100 Hz). Outward current plotted upward; thus, because the inner segment was within the electrode, an upward deflexion corresponds to an inward current across the outer segment membrane. The electrode was filled with normal toad saline (1·0 mM-calcium). Periodic saturating flashes of 500 nm light were given at times indicated by upward ticks on the top trace; flash duration was 20 ms, and the intensity was 120 photons μm^{-2}. During the heavy bar on the top trace the outer segment was perfused with calcium-free saline. Upon exposure to calcium-free saline, the dark current increased to about 200 pA, then began to decline. Graph beneath traces shows power spectral density of the dark noise during calcium-free perfusion, obtained as the difference between the spectrum from twenty-nine samples in normal saline and the spectrum from thirteen samples during perfusion. Data were digitized in 512-point samples at 1 ms point^{-1} (band width: 1–300 Hz). Data points are averages of two frequencies below 100 Hz and five frequencies above 100 Hz. The line was fitted to the data by eye and was drawn according to eqn. (1) with f_c = 39 Hz.

RESULTS

Effect of low calcium concentration on dark current

Noise upon initial exposure to low calcium concentration

The effect of briefly perfusing the outer segment with saline containing no added calcium and 2 mM-EGTA (i.e. calcium-free) is shown in the records of Fig. 2. In this experiment, the inner segment of an isolated rod was drawn into a suction electrode filled with normal saline, and the protruding outer segment was perfused with calcium-free saline via a second pipette placed nearby. As reported previously (Yau

et al. 1981; Hodgkin *et al.* 1984), the dark current increased dramatically upon exposure to the calcium-free saline, then declined toward a lower plateau level. In the experiment of Fig. 2, the initial dark current, measured from the peak of the saturating light response, was about 200 pA at the start of the perfusion pulse. When the calcium-free saline was removed, the dark current was at first completely suppressed and then recovered over a course of several minutes (Yau *et al.* 1981; Yoshikami, Foster & Hagins, 1983). During the calcium-free perfusion, the large dark current was accompanied by a pronounced increase in base-line noise. This can perhaps best be seen in the higher-gain, a.c.-coupled bottom trace of Fig. 2. Note that the noise was suppressed during the saturating light response triggered during calcium-free perfusion, and that the noise variance decreased as the dark current declined.

To examine the frequency composition of the noise accompanying calcium-free perfusion, its power spectral density was obtained by subtracting the power spectrum of segments of dark current in normal saline from the power spectrum of the dark current during initial exposure to calcium-free saline. For the experiment of Fig. 2, this difference spectrum is shown in the graph beneath the records. The line through the data points was drawn according to the expression for a single-Lorentzian power spectrum:

$$S(f) = S(0)/[1 + (f/f_c)^2], \quad (1)$$

where $S(f)$ is power spectral density, $S(0)$ is the zero-frequency asymptote, f is frequency, and f_c is the half-power, or corner, frequency. For the cell of Fig. 2, f_c was 39 Hz, corresponding to an exponential time constant of 4·1 ms. The corner frequency from samples of dark noise taken early during perfusion was similar to that of later samples, indicating that the spectral composition of the noise did not change during the decline in dark current. Results similar to those in Fig. 2 were also obtained in experiments in which the outer segment was exposed to calcium-free solution by transferring an isolated rod from normal saline to calcium-free saline, using the procedure described in the Methods (Fig. 1). In eleven cells, the difference power spectrum of noise during initial exposure to calcium-free saline was fitted by a single-Lorentzian equation, with f_c averaging $40{\cdot}1 \pm 9{\cdot}5$ Hz (mean $\pm$ S.D.). This corresponds to an average time constant of $4{\cdot}3 \pm 1{\cdot}5$ ms.

Noise following bright-flash responses in calcium-free saline

After the dark current had declined to a stable value during maintained exposure to calcium-free saline, saturating flash responses were often followed by a transient increase in dark current lasting 30–100 s. An example of such a rebound is shown in the top trace of the inset in Fig. 3, which shows results from an isolated rod that was transferred from normal to calcium-free saline. During the period of increased current, there was a pronounced increase in noise similar to that seen upon initial exposure to calcium-free saline. The noise can be seen more clearly in the a.c.-coupled middle trace in Fig. 3. This noise was also abolished during a saturating light response. The power spectral density of the noise was obtained by subtracting the spectrum of dark current before and after the rebound period from the power spectrum of segments of dark current near the peak of the rebound period. An example of the resulting difference spectrum of the noise is given in Fig. 3. In twenty-seven rods,

the difference spectrum of the noise was fitted by eqn. (1) with f_c of 29·7 ± 6·6 Hz (mean ± S.E.), corresponding to an average time constant of 5·6 ± 1·2 ms for the underlying shot-events. Thus, the noise following bright flashes was similar to the noise upon exposure to calcium-free saline, although the lower corner frequency for the rebound noise (29·7 *vs.* 40·1 Hz) suggests that shot-events were somewhat briefer during the large dark current immediately after exposure to calcium-free saline.

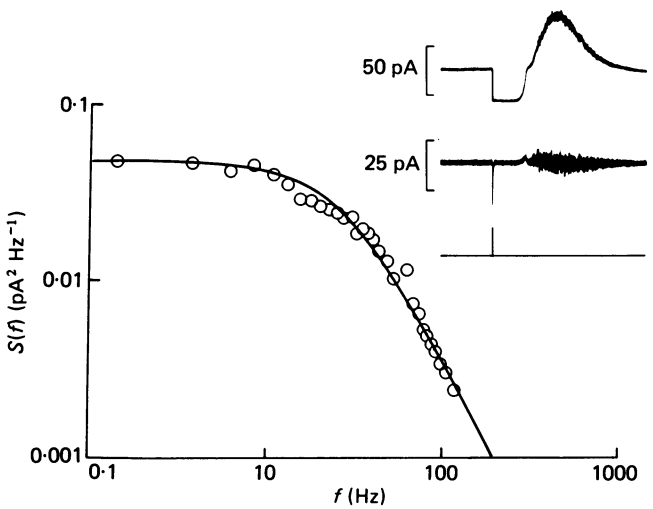

Fig. 3. Power spectrum of noise accompanying the rebound increase in dark current following a saturating flash in a rod whose outer segment was exposed to calcium-free saline. The inset shows sample chart recorder traces illustrating the rebound. The upward tick in the lower trace of the inset shows the timing of a 20 ms 500 nm flash of intensity 1300 photons μm^{-2}. The upper trace shows the change in membrane current, with outward current upward. Following the bright flash, the dark current (inward at the outer segment) was transiently suppressed. When the current reappeared, it transiently overshot the resting level and the variance increased. Middle trace in the inset is an a.c.-coupled version (band width 1–70 Hz) of the upper trace. The power spectrum in the graph was obtained from 512-point samples of dark current digitized at 2 ms point^{-1} with band width 1–150 Hz. The spectrum from samples of dark current before and after the period of increased current following bright flashes was subtracted from the spectrum during the increased current. Data were smoothed by averaging over five frequency points below 40 Hz, ten points in the range 40–100 Hz, and twenty-five points above 100 Hz. The line was fitted to the points by eye and was drawn according to eqn. (1) with f_c = 29 Hz. The length of each trace in the inset is 80 s.

Role of voltage changes in generation of noise

A single-Lorentzian power spectrum of membrane current noise, like those in Figs. 2 and 3, can arise directly from fluctuations in the ionic channels carrying the membrane current (Katz & Miledi, 1972; Anderson & Stevens, 1973; Neher & Stevens, 1977). Alternatively, a single-Lorentzian spectrum can arise from voltage fluctuations shaped by the membrane time constant of the cell (Katz & Miledi, 1972). In the present experiments, it is not clear whether the observed noise is related directly to the increased dark current, as would be the case if it arose in the

light-sensitive channels themselves, or indirectly in response to voltage changes associated with the increased dark current. The latter might occur, for example, if fluctuations in a current source other than the light-sensitive conductance gave rise to intracellular voltage fluctuations that, in turn, produced the noise in the membrane current recorded by the suction electrode.

Noise in rods attached to pieces of retina

If the source of the noise is voltage fluctuations, then attenuating voltage changes associated with the period of increased dark current should reduce or abolish the noise. To test this notion, suction electrodes were filled with calcium-free saline and recordings were made from outer segments of rods that remained attached to pieces of retina. The external bathing solution was normal saline. In this configuration only the outer segment of the recorded rod was exposed to calcium-free saline, the other rods in the piece remaining in normal saline. Rods in small pieces of retina are electrically coupled (Nunn, Matthews & Baylor, 1980), which should attenuate any voltage changes in the recorded rod accompanying the rebound increase in dark current following a bright flash. If the rebound noise depends on voltage changes, it too should be attenuated. Sample results from an experiment of this kind are shown in Fig. 4*A*. Note that in this Figure the response polarities are reversed from previous Figures because the outer segment, rather than the inner segment, was inside the suction electrode. With this recording configuration, pronounced increases in dark current after a saturating light response could still be seen, but there was little increase in variance associated with the increased current. In sixteen such experiments, the average percentage change in variance at the peak of the period of increased dark current was $4{\cdot}9 \pm 9{\cdot}9\,\%$ (mean $\pm$ S.D.; range from $-4{\cdot}9$ to $29{\cdot}8\,\%$).

In experiments like that of Fig. 4*A*, a small amount of normal saline from the bathing fluid necessarily entered the suction electrode while the outer segment was first entering the electrode; therefore, the calcium concentration bathing the outer segment inside the electrode was probably somewhat higher than the calcium concentration bathing the outer segment in experiments described earlier, in which isolated rods were transferred to calcium-free saline. This difference could possibly account for the failure to observe a noise increase in attached rods. To examine this possibility, similar recordings using suction electrodes filled with calcium-free saline were made from the outer segments of isolated rods. Unlike attached rods, isolated rods showed clear noise increases during the rebound increase in dark current, as illustrated in Fig. 4*B*. In experiments on isolated rods, the average increase in variance at the peak of the post-light increase in dark current was $102 \pm 96\,\%$ (mean $\pm$ S.D., $n = 25$; range 7·8–351 %). Thus, the lack of a noise increase in attached rods was not due to the recording configuration.

Effect of caesium on noise in low calcium concentrations

Results of the previous section suggest that voltage fluctuations are important in generating the noise in outer segment membrane current. One source of such fluctuations might be voltage-sensitive channels of the inner segment, such as the caesium-sensitive conductance activated by hyperpolarization (Fain, Quandt, Bastian & Gerschenfeld, 1978; Bader, Bertrand & Schwartz, 1982). The rebound period of

increased dark current following a saturating light response (Figs. 3 and 4) might reflect activity of the electrogenic sodium–potassium pump (Torre, 1982), in which case the membrane potential might hyperpolarize during the rebound into a range in which the caesium-sensitive mechanism is active. To examine the possible role of

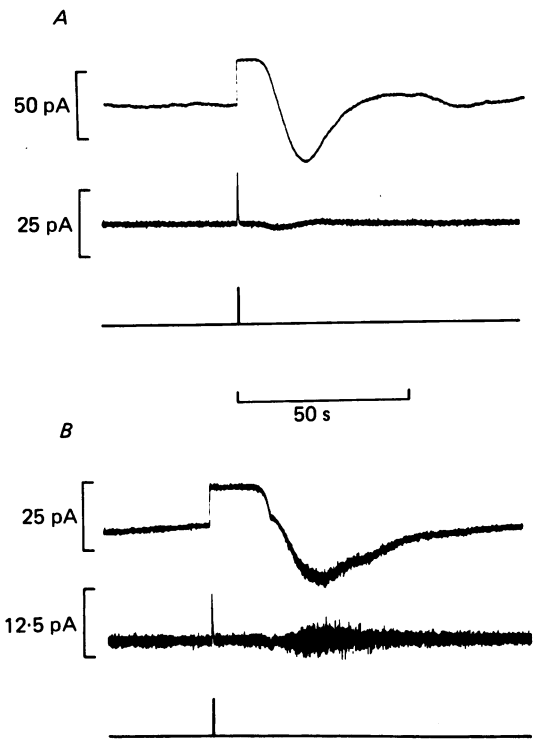

Fig. 4. Increased dark current following bright flashes in a rod attached to a piece of retina (*A*) and an isolated rod (*B*). In both cases, the outer segment was drawn into a suction electrode containing calcium-free saline, and the fluid outside the electrode was normal saline. The upper traces show membrane current with outward current plotted upward (band width: 0–40 Hz), the middle traces are high-pass filtered versions of the upper traces (band width: 1–40 Hz), and the lower traces show timing of 20 ms 500 nm flashes of intensity 4200 photons μm^{-2}. In both cells, there was a pronounced increase in dark current after a saturating light response, but an accompanying increase in noise occurred only in the isolated cell.

this conductance in the generation of the noise, suction electrodes were filled with calcium-free saline and recordings were made from outer segments of isolated rods, as in the previous section. Saline containing 10 mM-CsCl (replacing 10 mM-NaCl) was then passed over the protruding inner segment by means of a separate perfusion pipette placed at a right angle to the recording suction electrode (Liebman *et al.* 1984). Caesium perfusion may also interfere with potassium channels (Torre, 1982), which might play a role in generating voltage fluctuations. Results from one experiment of this type are shown in Fig. 5. During caesium perfusion (Fig. 5*A*), the rebound

increase in dark current was observed following a saturating light, but there was little associated increase in variance. When the caesium perfusion was halted, the usual noise increase was seen (Fig. 5*B*). For the cell of Fig. 5, the peak variance (relative to that during a saturating light in normal saline) was 0·34 pA^2 (band width 1–150 Hz)

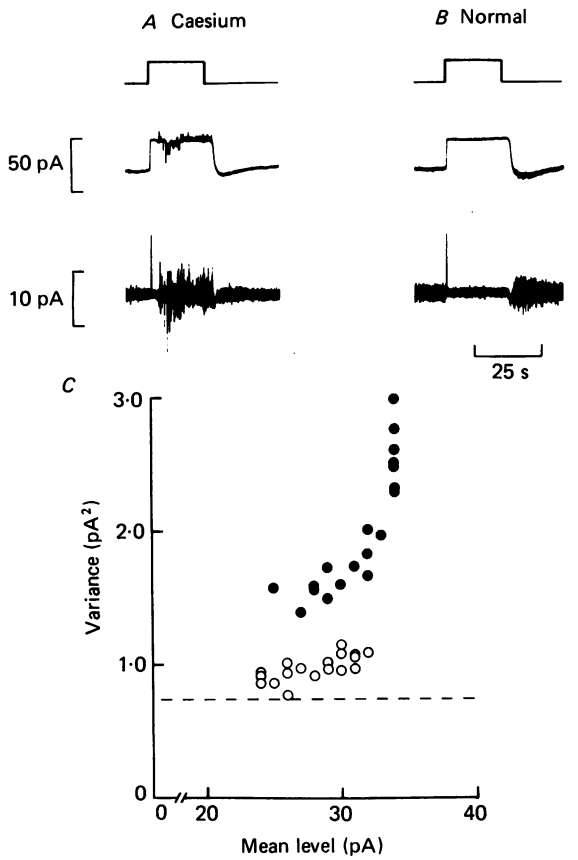

Fig. 5. Noise following saturating light during (*A*) and after (*B*) caesium perfusion. Caesium (10 mM) was applied to the inner segment by local perfusion. Top traces show timing of saturating 500 nm light of intensity 2600 photons μm^{-2} s^{-1}. Middle two traces show membrane current at band widths of 0–40 and 1–40 Hz and at two different gains. *C*, relation between variance and mean level of dark current during the rebound periods in *A* (○) and *B* (●). Variance was calculated from 512-point samples of dark current digitized at 2 ms intervals and a band width of 1–150 Hz. The mean level of the dark current was measured from the plateau of the saturating light response. The dashed line indicates the variance during saturating light in the absence of caesium.

during caesium perfusion and 1·74 pA^2 in the absence of caesium. Similar results were observed in eleven other cells.

In the cell of Fig. 5, but not in all cells, the rebound increase in dark current was slightly smaller in the presence of caesium: the rebound reached a peak of 32 pA with caesium and 34 pA without. This raises the question of whether the reduction in variance by caesium might be caused by the reduction in dark current. However, this

was not the case, as demonstrated by the data in Fig. 5*C*, which show that at comparable levels of dark current the variance in caesium was less than in normal saline. Thus, caesium reduced the steepness of the relation between variance and amplitude of the dark current during the rebound period.

TABLE 1. Effect of caesium perfusion during the rebound increase in dark current following a saturating light. To eliminate instrumental noise, the variance during a saturating light was subtracted from the measured dark variance to give the listed values. Band width for variance calculation was 1–150 Hz. Dark current was measured from the level during saturating light and was corrected for perfusion artifact in the case of caesium. Perfusion artifact was estimated as described by Liebman *et al.* (1984). Caesium perfusion was given at about the peak of the post-light rebound. Abbreviations: σ_I^2 and J_I are variance and mean level of dark current before caesium perfusion, and σ_{Cs}^2 and J_{Cs} are variance and mean during caesium perfusion

Experiment	σ_I^2 (pA^2)	σ_{Cs}^2 (pA^2)	σ_{Cs}^2/σ_I^2	J_I (pA)	J_{Cs} (pA)	J_{Cs}/J_I
1	0·77	0·14	0·18	30	28	0·93
2	1·41	0·17	0·12	50	46	0·92
3	0·61	0·11	0·18	32	28	0·88
4	0·26	0·01	0·04	40	37	0·93
5	1·66	0·22	0·13	42	40	0·95
6	0·96	0·14	0·15	25	23	0·92
7	1·19	0·54	0·45	54	56	1·04

It is possible that the effect of caesium resulted from caesium leaking into the interior of the pipette and acting directly on the outer segment to reduce the rebound noise. The membrane of the outer segment has little residual conductance when all light-sensitive channels are closed in bright light (Baylor & Lamb, 1982; Baylor & Nunn, 1983), i.e. it appears to contain only light-sensitive channels. Thus, a direct action of caesium on the outer segment would be mediated via the light-sensitive channels themselves. In this view, a large reduction in the variance of the dark current during the rebound period should be accompanied by a large reduction in the magnitude of the dark current. However, Yau *et al.* (1981) reported that caesium applied to the outer segment of toad rods had no blocking action on the dark current; thus, a direct action of caesium on the outer segment is unlikely. Nevertheless, this alternative was examined by abruptly perfusing the inner segment with caesium when the post-light increase in dark current was at its peak. The variance of the dark current was calculated just before and just after onset of the caesium perfusion, and results from seven experiments are listed in Table 1. As expected from Fig. 5, caesium dramatically reduced the variance. The change in dark current produced by caesium perfusion was also estimated, although this was complicated by the presence of an artifactual base-line shift caused by the perfusion (Liebman *et al.* 1984; Hodgkin *et al.* 1984). To isolate the change in dark current, the size of the perfusion artifact was estimated either by giving a brief caesium perfusion during a steady saturating light or by measuring the caesium-induced shift in the d.c. level reached in response to a saturating light flash (Liebman *et al.* 1984). The resulting estimates of the effect of caesium on the dark current are also given in Table 1. In all experiments but one, caesium produced a small decrease in the dark current, possibly due to a reduction in inner segment conductance. In the other experiment, there was a small apparent

increase in the dark current, although this probably reflects error in estimating the perfusion artifact. The reduction in variance was much greater than would be expected from the small decrease in dark current, suggesting that the effect of caesium on the noise is not mediated via an effect on the light-sensitive current. A simple alternative is that caesium acts on a current source other than the light-sensitive current, such as conductance mechanisms of the inner segment. In the latter view, the caesium-sensitive current source sets up noise in the dark current indirectly via fluctuations in intracellular voltage.

Bursts of noise during the response to saturating light were frequently observed during caesium perfusion (see Fig. 5*A*, for example). Such noise during bright light in caesium-containing saline has been observed previously in experiments using intracellular recording by Torre (1982), who suggested that this noise may be due to reversible dielectric breakdown of the membrane at the high levels of polarization (-100 mV or greater) reached under such conditions. The effect of caesium on the post-light rebound noise might be due to possible damaging effects of such breakdown. However, this possibility was excluded in the experiments in which caesium perfusion was restricted to the period of rebound noise (Table 1), in which case the 'breakdown' during the light did not occur. In this situation, caesium attenuated the rebound noise as it did during steady perfusion.

DISCUSSION

Fluctuations in the outer segment membrane current like those reported here could arise from conductance fluctuations in the light-sensitive conductance of the outer segment membrane or from voltage fluctuations produced by current sources elsewhere in the cell. The observed single-Lorentzian spectra could result from an exponential distribution of duration of unitary conductance events (Neher & Stevens, 1977); alternatively, voltage shot-events shaped by the input time constant of the cell could produce the same sort of spectrum (Katz & Miledi, 1972). Because internal voltage was not controlled in the experiments reported here, no direct evidence was obtained on this question; however, results of experiments with rods attached to retinal pieces and with caesium suggested that the noise arose from voltage fluctuations. In this view, the observed fluctuations in light-sensitive current reflect fluctuations in the intracellular voltage driving that current.

Interpretation of corner frequency of noise in low calcium concentrations

If it is correct that the membrane current noise measured with the suction electrode arises from voltage fluctuations, if the voltage fluctuations are small, and if voltage-sensitivity of the light-sensitive channels themselves is ignored, then the corner frequency of the single-Lorentzian power spectrum would correspond to the membrane time-constant of the isolated rod. Assuming specific membrane capacitance of 1 μF cm^{-2} and assuming input resistance similar to that of isolated salamander rods (1 GΩ, Baylor, Matthews & Nunn, 1984), the membrane time constant of isolated toad rods would be about 13 ms. This is somewhat longer than the average values of 5·6 ms for the time constant of noise following saturating light responses and 4·3 ms for the time constant of noise immediately after entering calcium-free

saline (see p. 210). The fact that the observed time constants were briefer than the anticipated time constant might indicate a lower input resistance in calcium-free saline, as expected if the outer segment conductance was higher in the calcium-free solution. Similarly, the shorter time constant (4·3 *vs.* 5·6 ms) for noise immediately after entering calcium-free saline may indicate that the outer segment conductance during the period of large dark current (> 200 pA) was higher than after the dark current had declined to a steady value. Such a reduction in outer segment conductance with time would be consistent with the notion that sodium accumulates inside a rod exposed to low external calcium and that calcium extrusion via sodium–calcium exchange is retarded as a result, leading to an increase in internal calcium and the closing of some light-sensitive channels (Yau *et al.* 1981). The time constant of the noise did not appear to change during the rapid decline in dark current after initial exposure to calcium-free saline (see p. 209), suggesting that the putative decline in conductance in the calcium-free saline occurs more slowly than the initial relaxation in the current.

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