HIGH-PASS FILTERING OF SMALL SIGNALS BY RETINAL RODS

Ionic Studies

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ABSTRACT The high-pass filtering of small signals by the rod photoreceptor network was studied by intracellular recording in the isolated, perfused retina of the toad, *Bufo marinus*. Data were analyzed and interpreted in terms of the network analysis described in the preceding paper. External concentrations of Cs^+ as high as 10 mM, which blocked the relaxation from peak to plateau of the rod's response to bright light, did not affect the filtering of small signals. The effects of reducing $[Na^+]_o$ were not consistent with a direct action upon the mechanism underlying this filtering property. By contrast, raising external $[K^+]$ from 2.6 to 10 mM, which caused a fourfold reduction in E_K, abolished the high-pass filtering of small signals. Analysis of the effects of external $[K^+]$ changes indicates that the underlying mechanism involves a K^+ conductance that decreases with a delay when the rod is hyperpolarized. This conductance is not blocked by externally applied tetraethylammonium. Other experiments did not rule out the possibility that it might be activated by Ca^{++}.

INTRODUCTION

In the preceding paper (Torre and Owen, 1983) we described experiments designed to characterize the high-pass filtering of small signals by the network of rod photoreceptors in the retina of the toad, *Bufo marinus*, and presented a theoretical description of the phenomenon based upon an equivalent circuit model. It was noted that high-pass filtering might be accounted for by three different mechanisms and that our theoretical treatment did not distinguish between them. Our purpose, in the present paper, is to describe experiments in which the ionic environment of the photoreceptors was changed with a view to identifying the site and nature of the mechanism underlying the high-pass filtering behavior.

The plasma membrane of the rod is known to contain time-varying, voltage-dependent conductances that profoundly affect the time course of the rod's voltage response to light. Blockage of one such conductance by low concentrations of Cs^+ eliminates the marked relaxation from peak to plateau of the voltage response to a near-saturating, diffuse light (Fain et al., 1978). Recently, it was shown that the properties of voltage-gated currents measured in isolated rods can give rise to high-pass filtering by the rod network (Attwell and Wilson, 1980).

In a recent paper, Capovilla et al. (1981) showed that in the toad, *Bufo bufo*, the time-to-peak of the rod's voltage response to a weak, diffuse flash of light increased by 30–40% when the retina was perfused with 10 mM K^+. This was accompanied by a depolarization of the membrane potential in darkness by 9 mV. They were unable to determine whether this effect resulted from the change in the dark potential or from the reduction in the driving force on K^+.

In this paper we suggest that the high-pass filtering of small signals by the rod network of *Bufo marinus* is due to the action of a time-varying, voltage-dependent K^+ conductance that is not blocked by low concentrations of Cs^+, nor by 10–20 mM tetraethylammonium (TEA), but may be Ca^{++} dependent. A brief summary of some of these findings was given in an earlier publication (Torre and Owen, 1981).

RATIONALE

A reduction or abolition of the high-pass filtering behavior can be accounted for by the network model in three different ways (Torre and Owen, 1983, Fig. 13). The inductance can be shunted, either by increasing g_1 or by decreasing R_s, or it can be blocked by decreasing g_2. It goes without saying that the inductance can have an effect only so long as current flows through it. Thus, we might block the inductance either with a true blocking agent or by

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reducing the driving force on the ion carrying the current through the inductance so that current becomes negligible. The effect in either case would be registered as a reduction in the value of g_2. The true cause of that reduction should be clear from the experimental protocol. Our criterion for a specific effect upon the mechanism responsible for high-pass filtering, therefore, was that there should be a significant decrease in the value of g_2 of the network equivalent circuit.

METHODS

Because it appeared likely that high-pass filtering by the rod network depends upon the modulation of an ionic conductance, either as a direct result of a change in membrane voltage or by the action of a synaptic transmitter released between neighboring rods, our strategy was to study the effects of changing the rod's external environment. By fitting theoretically generated responses to the recorded data, we planned to establish which of the parameters of the network equivalent circuit (Torre and Owen, 1983) were changed by exposure to each test solution and to interpret these changes in terms of ionic currents.

Apparatus and recording techniques were described in the preceding paper (Torre and Owen, 1983). Most of the experiments involved the presentation of a narrow, slit-shaped stimulus at successive 10-μm displacements from the impaled rod. The rationale and basic protocol of this type of experiment were described in the preceding paper. In the present study, however, it was necessary to change the ionic concentrations of the perfusion medium. In each experiment, therefore, a sequence was followed in which the rod was exposed in turn to control Ringer's solution, test solution(s), and control Ringer's solution once more. Only data from experiments in which responses were identical during both initial and final exposures to control Ringer's solution were used. For convenience, this type of experiment will be referred to throughout the remainder of this paper as the slit experiment.

For changing perfusion media, two different methods were used: (*a*) The perfusion media, a control Ringer's solution, and three test Ringer's solutions were stored at room temperature during the experiment in four bottles, where they were continuously bubbled with 95% O_2 and 5% CO_2. Each bottle was connected by a Teflon tube to one of the taps of a four-way manifold. A fifth tap allowed solutions to flow from the manifold to a waste bottle. Before the experiment a little of each solution in turn was flowed through the manifold to waste, thereby removing air bubbles from the system. Once the waste tap was closed, the chosen solution could be passed to the perfusion chamber by opening the appropriate tap, its flow rate being limited to ~2 ml/min by a needle valve. A change of solution could be effected with no fluctuation in flow rate by opening the tap of the new solution before closing that of the old. This technique was satisfactory in that it permitted recordings to be maintained through many solution changes, but suffered from a number of disadvantages. The principal problem was that the dead space of the system was large and more than a minute had to elapse before the new solution reached the chamber. Moreover, the inevitable mixing of solutions during the changeover added to the uncertainty in timing the concentration change in the chamber. An alternative system was devised to overcome these deficiencies.

(*b*) Each of six bottles was connected in parallel to two six-way taps. Thus, any two of the solutions could be passed to a third tap located inside the Faraday cage, close to the perfusion chamber. This latter tap had two efflux pathways, one that passed to a waste bottle, the other to the chamber. By turning the tap, either of the two chosen solutions could be passed to the chamber while the other drained into a waste bottle. The tap was designed so that the flow through the efflux tubes was not interrupted during switching. Once the solutions had been changed the solution flowing to waste could be stopped by turning the appropriate six-way tap to an intermediate off position. The flow rate was regulated by the bore of the tube connecting the final tap to the chamber. Because this was very narrow, the dead space in the system was minimal. The effect of switching solutions was seen within seconds and ionic concentrations in the chamber could be completely changed in less than half a minute. Similar results were obtained using both techniques.

RESULTS

Effect of Caesium Ions

It is known that the relaxation from the peak to the plateau of the rod's voltage response to a near-saturating, diffuse flash of light can be blocked by Cs^+ in concentrations as low as 2 mM (Fain et al., 1978). The voltage-dependent, time-varying conductance responsible for this relaxation has properties that might also account for high-pass filtering of small signals by the rod network (Attwell and Wilson, 1980). If these two phenomena shared a common mechanism, however, it would be expected that they should both be blocked by low concentrations of caesium.

Perfusion of the retina with concentrations of Cs^+ as high as 20 mM had a negligible effect upon the dark potential of the rods. The records shown in Fig. 1 *a* were obtained with control Ringer's solution. The shortening of the time-to-peak of the response as the slit-shaped stimulus was laterally displaced in successive 10-μm steps is clearly evident. We then switched to a medium to which 2 mM CsCl had been added and monitored the responses to near-saturating flashes of diffuse light to ensure that the peak-plateau relaxation had been blocked. Some two minutes later we once more presented the sequence of

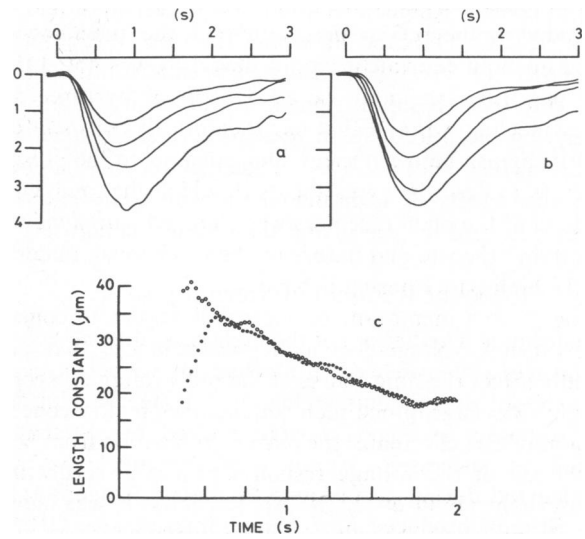

FIGURE 1 Negligible effect of Cs^+ on high-pass filtering. (*a*) Intracellular responses to a slit of light 11 μm × 1 mm flashed at displacements 0, 10, 20, and 30 μm from the impaled rod while perfusing with control Ringer's solution. (*b*) Experiment repeated in Ringer's solution containing 2 mM Cs^+. (*c*) Length constant of network (λ) plotted as a function of time from records in *a* and *b*. ●, 2 mM Cs^+; ○, Ringer's solution. All responses in this and subsequent figures were averaged and smoothed as described in the preceding paper (Torre and Owen, 1983).

displaced slit-stimuli and recorded the responses shown in Fig. 1 *b*. The shortening of the time-to-peak is again clearly evident, which indicates that the high-pass filtering by the rod network was unaffected by the presence of Cs^+.

In Fig. 1 *c* we have plotted the network length constant computed as a function of time during the response. Although there was some discrepancy between the values early in the response (Torre and Owen, 1983), the fall in length constant during the remainder of the response was essentially identical in both media. Analysis of voltage responses elicited by diffuse light flashes indicates that the effect of 2 mM Cs^+ becomes apparent only when the response amplitude is greater than 6–8 mV (Fain and Lisman, 1981; W. G. Owen and V. Torre, unpublished observations). Because the membrane potential in darkness (dark potential) was -44 ± 3 mV, (mean of 58 cells ± SEM), this suggests that the Cs^+-sensitive conductance is activated only at potentials more negative than ~ -50 mV and does not contribute to the shape of weak responses.

We cannot completely exclude an alternative explanation, however. The blocking action of Cs^+ might itself be voltage-dependent (Coronado and Miller, 1979; Ciani et al., 1980) such that a hyperpolarization beyond -50 mV is necessary before it becomes effective. We believe this explanation to be unlikely, however, because the addition of up to 10 mM Cs^+, a concentration that would be expected to compensate for the postulated voltage-dependence of the blocking action of Cs^+, had little effect upon the high-pass filtering behavior of the network. In the presence of 20 mM Cs^+, the time course of the response to diffuse illumination was, if anything, marginally faster than in control Ringer's solution, not slower as would be expected if Cs^+ blocked high-pass filtering. We propose, therefore, that the mechanism responsible for the high-pass filtering of small signals is different from that underlying the peak-plateau relaxation in the response to bright, diffuse stimuli. This suggestion receives support from the results of experiments in which the external concentration of K^+ was changed (see later sections).

Effect of Reducing External $[Na^+]$

To determine whether or not the high-pass filtering behavior involves a Na^+-dependent mechanism, we performed a series of slit experiments in low-Na^+ Ringer's solutions. The responses in Fig. 2 *a* were elicited by slit-shaped stimuli at successive 10-μm displacements from the impaled rod during perfusion with control Ringer's solution. Stimuli produced 65.7 photoisomerizations (Rh*)/flash, on average. The open circles in Fig. 2 *d* show that during the control Ringer's solution response the network length constant, λ, fell from 30 to 18 μm in this particular experiment.

When external Na^+ was reduced from 132 to 80 mM by substitution with lithium, the rod hyperpolarized by ~2.5 mV. We then repeated the slit experiment, obtaining the

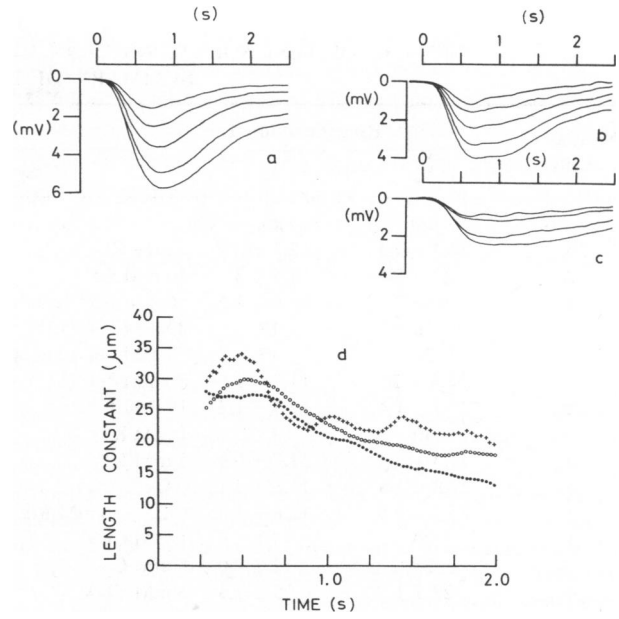

FIGURE 2 Effect of reducing $[Na^+]_o$ to 80 mM. (*a*) Responses elicited by flashed slit at displacements of 0, 10, 20, 30, and 40 μm from impaled rod while perfusing with control Ringer's solution. Experiment repeated in (*b*) with Ringer's solution containing 80 mM Na^+ and 52 mM Li^+ and in (*c*) with Ringer's solution containing 80 mM Na^+ and 52 mM choline. (*d*) λ plotted as function of time from records of (*a–c*). +, 80 mM Na^+ and 52 mM choline$^+$; ●, 80 mM Na^+ and 52 mM Li^+; ○, Ringer's solution.

responses shown in Fig. 2 *b*. The time course of the response was clearly affected, most noticeably during the return to dark potential following the peak. Plotting the network length constant as a function of time (filled circles in Fig. 2 *d*) clearly showed that λ fell during the response from ~27 to ~14 μm. The network still behaved as a high-pass filter, therefore.

Replacing the lithium with choline (80 mM Na^+, 52 mM choline) resulted in a further hyperpolarization of ~5.5 mV and a marked reduction in response amplitude. As seen in Fig. 2 *c*, the response time course was further slowed, the later portion of the response again being most markedly affected. The crosses in Fig. 2 *d* show that, although there may have been some reduction in the high-pass filtering behavior of the network in this case, it clearly was not abolished. In four experiments in 80 mM Na^+ (choline), the length constant, λ, fell on average from ~28 to ~15 μm during the response (Table I). Reducing $[Na^+]_o$ still further to 50 mM by substituting lithium in the presence of 2 mM Cs^+ caused a reduction in the early value of λ to ~21 μm. During the response, however, there was again a marked fall in λ to ~12 μm (Table I).

In the preceding paper (Torre and Owen, 1983), analysis of the network model showed that a reduction in the initial value of λ, with little effect upon the final value, is what would be expected if the conductance, g_l, in parallel with the arm of the circuit containing the inductance, were

TABLE I
EFFECTS OF TEST SOLUTIONS UPON HIGH-PASS FILTERING BY THE ROD NETWORK: SUMMARY OF DATA FROM 104 RODS

Number of cells	Control Ringer's solution		Test solution			
	λ_{in}	λ_{ss}	Test solution	ΔVm	λ_{in}	λ_{ss}
	μm	μm		mV	μm	μm
3	24.3 ± 4.3	12.3 ± 2.9	2 mM Cs^+	0	26 ± 3.5	14.3 ± 2
3	27 ± 5	14.5 ± 5	10 mM Cs^+	0	27 ± 3	17.5 ± 1.5
4	30 ± 2	15.6 ± 1.5	80 mM Na^+ (choline)	−8.2 ± 1	27.7 ± 1.3	15.3 ± 1.8
1	30	17	80 mM $Na^+(Li^+)$	−2.5	29	12
1	27	15	50 mM Na^+(choline)	−13	20	15
4	21.4 ± 2	11.7 ± 1.5	50 mM $Na^+(Li^+)$ + 2mM Cs^+	−5	19 ± 0.5	14 ± 2.0
22	22.7 ± 1.4	12.3 ± 1.3	10 mM K^+	+1.3 ± 0.5	18.2 ± 0.98	18.35 ± 1.0
1	30	15	1 mM K^+	−1	30	14
3	24.3 ± 2.3	11.7 ± 0.9	5 mM K^+	+0.7 ± 0.3	22.7 ± 3.7	14.3 ± 2
5	27.8 ± 1.8	11.8 ± 3.1	0 Ca^{++}	+27.2 ± 4.2	14 ± 1.7	13.8 ± 1.5
7	25.2 ± 2.5	12.6 ± 1.3	0 Ca^{++}, 6 mM Mg^{++}, 50 mM Na^+	+4.2 ± 1	13.4 ± 1.2	16.5 ± 1.3
1	20	10	1 mM Ca^{++}	+6	25	8
2	25	12 ± 2.0	5 mM Ca^{++}	−7	22.5 ±	9 ± 1
4	28 ± 1	14.75 ± 1.3	5 mM TEA	+5.7 ± 6	35.7 ± 2	17.2 ± 2
1	18	11	15 mM TEA	+10	42	12
4	26 ± 3	14.2 ± 1.3	15 mM TEA + 10 mM K^+	+12 ± 11	30 ± 2	21 ± 1.5
4	20 ± 1	10.5 ± 0.5	15 mM TEA + 15 mM K^+	+12 ± 2	20.7 ± 3	20.3 ± 0.5
2	27	15.5 ± 1.3	15 mM TEA + 20 mM K^+	+17.5 ± 2	23 ± 1.5	25 ± 1
4	33.7 ± 2.5	19.3 ± 4.2	15 mM TEA + 200 μM Co^{++}	+1	72 ± 1.5	32 ± 8
21	25.8 ± 1.4	13.9 ± 1.2	700 μM Co^{++}	−6 ± 1	20.6 ± 1.5	16.8 ± 1.2
3	25 ± 1	12 ± 1.5	5 mM Cs^+ + 1 mM CO^{++}	−6 ± 0.5	27.6 ± 2.4	15.7 ± 2.5
3	26.6 ± 2	14.3 ± 1.5	5 mM Na aspartate	−2	28 ± 1	15 ± 1.3
1	30	17	5 mM Na glutamate	−1	26	14

significantly increased. Our results with low $[Na^+]_o$ are thus consistent with an increase in membrane conductance. In view of this, we conclude that the high-pass filtering of small signals does not depend upon the properties of channels that are significantly permeable to Na^+ ions.

Effect of Increasing External $[K^+]$

To determine whether K^+ ions play an important role in the high-pass filtering behavior of the rod network, we compared the results from a series of slit experiments performed in control Ringer's solution with those obtained when the perfusate contained 10 mM K^+. Surprisingly, on raising external $[K^+]$ from 2.6 to 10 mM, the rod's dark potential depolarized on average by <2 mV. Response amplitudes decreased markedly, however, and it was necessary to increase stimulus intensity in the high $[K^+]_o$ Ringer's solution to elicit responses of comparable size with those recorded in control Ringer's solution.

Fig. 3 *a* and *b* show responses recorded when the experiment was performed while perfusing with control Ringer's solution and with 10 mM $[K^+]$ Ringer's solution, respectively. Note that on switching to the high $[K^+]_o$ medium, the time course of the responses became slower. The time-to-peak of this rod's response to a centered slit increased from 580 to 710 ms, despite an increase in stimulus intensity from 28.7 to 65.7 Rh*/flash. A slowing of the response time course and increase in time-to-peak in high $[K^+]_o$ was consistently observed in all rods tested. More significant, perhaps, is that in the presence of 10 mM K^+, the shortening in the time-to-peak with displacement of the stimulus no longer occurred, suggesting that the high-pass filtering behavior had been eliminated.

Fig. 3 *d* shows that in control Ringer's solution, (open circles) the length constant of the rod network fell in this case from 27 to 12 μm during the response. In the presence of 10 mM K^+, the length constant remained at ~21 μm, which confirms that the network no longer behaved as a high-pass filter to laterally propagating small signals. One possible explanation for this effect depends upon the fact that the measured length constant of the network is a function of stimulus frequency (Torre and Owen, 1983; Fig. 13). It was thus possible that raising external $[K^+]$ rather than directly diminishing the effect of the inductive reactance had merely slowed the time course of the response sufficiently so that the appropriate value of the length constant, at all times, had become the low frequency value. Although the length constant observed in 10 mM K^+ was closer to the high-frequency value than to the low-frequency value, we tested this possibility by repeating the experiment in the presence of steady background illumination that produced, on average, 4.3 Rh*/s in each rod. This reduced the sensitivity of the rods by a factor of ~2, and it was necessary, therefore, to increase stimulus

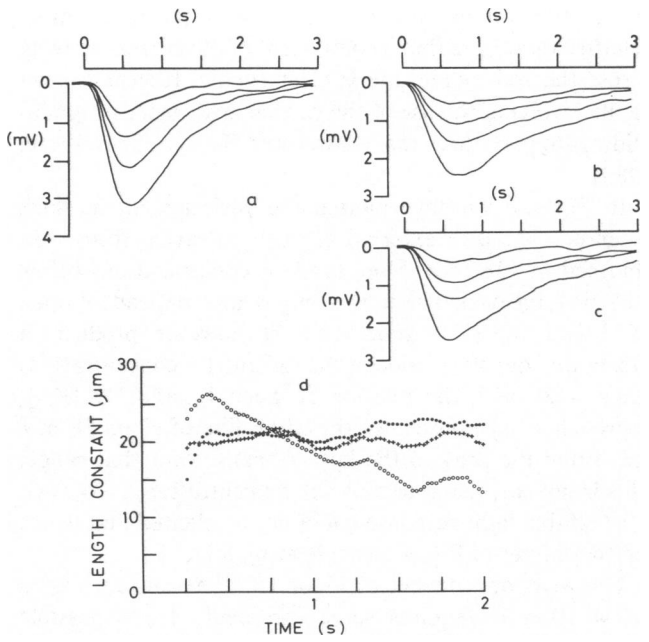

FIGURE 3 Effect of raising $[K^+]_o$ to 10 mM. (*a*) Responses elicited by displaced stimuli while perfusing with control Ringer's solution. Stimulus intensity = 28.7 Rh*/flash. (*b*) Experiment repeated in Ringer's solution containing 10 mM$[K^+]_o$. Stimulus intensity = 65.7 Rh*/flash. (*c*) Experiment in 10 mM$[K^+]_o$ repeated against an illuminated background (4.3 Rh*/s). Stimulus intensity = 137.3 Rh*/flash. (*d*) λ plotted as a function of time from each of the three sets of records. ●, 10 mM K^+; +, 10 mM K^+ background; O, Ringer's solution.

intensity to 137.3 Rh*/flash to elicit responses of comparable amplitude to those of Fig. 3 *b*. As seen in Fig. 3 *c*, an effect of the background was to quicken the time course of the responses so that their times-to-peak were comparable with those measured in control Ringer's solution. Note, however, that despite this, the time-to-peak was no longer dependent upon stimulus position. The crosses in Fig. 3 *d* show that the length constant remained fixed at 20 μm throughout the response, and thus was not significantly affected by the presence of the background. We therefore discount this explanation of the effect of 10 mM K^+.

Neither do we believe that the high-pass filtering was abolished as a result of the small depolarization produced by perfusing with 10 mM K^+. In the present study the average depolarization was 1.3 mV ± 0.3 (SEM of 36 rods). Moreover, in the experiment shown in Fig. 3, although perfusion with 10 mM K^+ caused a 3-mV depolarization in darkness, the background illumination produced a hyperpolarization of the same magnitude. Thus, the responses of Figs. 3 *a* and *c* were recorded at the same baseline potential. The disappearance of high-pass filtering in the presence of 10 mM K^+ cannot therefore be attributed to the depolarization of the membrane.

Complete experiments were carried out on 22 rods. The collected data from these experiments show that in control Ringer's solution the network length constant declined during the response from 22.7 to 12.4 μm. In 10 mM K^+ the length constant remained unchanged at ~18 μm. Experiments in which $[K^+]_o$ was raised to 5 mM showed that this lower concentration reduced high-pass filtering by the rods but did not abolish it. To summarize, therefore, raising $[K^+]_o$ to 10 mM abolished high-pass filtering by the rod network. This was not due to the observed slowing of the response time course nor to the small depolarization in the rod's dark potential.

K^+ System in the Rod

As mentioned in the Rationale, our analysis of the network equivalent circuit revealed that high-pass filtering could be abolished either by increasing the parallel conductance, g_1, or by decreasing the conductance, g_2, in series with the inductor. The first case would correspond to a shunting of the time-dependent mechanism, the latter to blocking it. If we suppose that the high-pass filtering is due to a voltage-dependent K^+ conductance that obeys first-order kinetics, we can write

$$\dot{g}_K = \frac{g_K^\infty(V) - g_K}{\tau_K(V)}, \tag{1}$$

where $g_K^\infty(V)$ is the voltage-dependent value of the K^+ conductance at $t = \infty$, g_K is its instantaneous value at time t, and τ_K is the time constant. The conductance g_2 of the circuit in Fig. 2 *a* will be given by

$$g_2 = (V - E_K)\frac{\partial g_K}{\partial V}\bigg|_{\bar{V}}, \tag{2}$$

where g_K is the K^+ chord conductance and $\bar{V}$ the resting potential in darkness. We see that g_2 may be reduced by changing E_K. If the effect of raising $[K^+]$ to 10 mM were simply to reduce g_2, however, we would expect the network length constant to remain fixed at its initial (high frequency) value throughout the response. Experimentally we observe that λ remains fixed at a value slightly lower than the high-frequency value in control Ringer's solution. This can only be explained if 10 mM K^+ causes changes in the values of other network parameters, too; in particular, the values of g_1 and R_s. It was therefore necessary to perform a more detailed series of experiments to characterize the effects of changing $[K^+]_o$ on both the dark potential and the light-evoked voltage response of the rod.

We first carried out an experiment to estimate the equilibrium potential for K^+, (E_K). This was based on the argument that in the presence of 2 mM Cs^+, which blocks the peak-plateau relaxation, the peak of the response to a saturating stimulus should approach E_K. The assumptions are that at potentials below −60 mV, in the presence of Cs^+, any remaining voltage-gated currents will have been inactivated (Attwell and Wilson, 1980), and that following a saturating flash, all Na^+ channels in the membrane are closed.

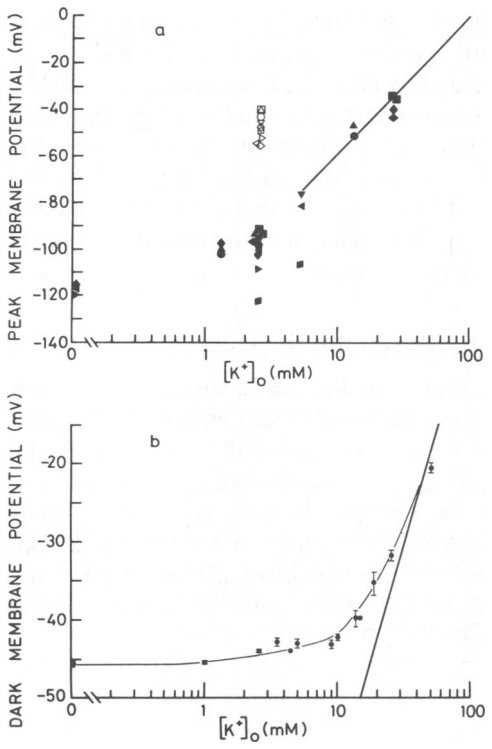

FIGURE 4 Effect of varying $[K^+]_o$ upon membrane potential in darkness and at peak of the response to a diffuse, saturating light flash in the presence of 2 mM Cs^+. (*a*) Peak membrane potential elicited by a diffuse stimulus of intensity 6,200 Rh*/rod/flash (filled symbols). Data from 10 rods. Open symbols indicate the dark membrane potential in control Ringer's solution. The solid line has a slope of 58 mV/decade. (*b*) Dark membrane potential plotted as a function of $[K^+]_o$. Data from 136 rods. Error bars plot the standard error of the mean at each concentration. The straight line that forms an asymptote with the data is the same line as is drawn through the data of *a*.

In Fig. 4 *a* are plotted data collected from 10 different rods. The open symbols plot the dark potentials measured in control Ringer's solution (2.6 mM K^+) to which 2 mM Cs^+ had been added. The filled symbols plot the potentials at the peaks of responses to diffuse flashes of light that produced, on average, 6,200 Rh*/flash in each rod. The solid line through the data at K^+ concentrations above 5 mM was fitted by eye and has a slope of 58 mV/decade. The fact that it is an acceptable fit to the data indicates that at high concentrations of K^+, the rod membrane approximates the behavior of a K^+ electrode.

If we make the worst-case assumption that the only free cation inside the rod is K^+ then, given that the rod is in osmotic equilibrium, the K^+ equilibrium potential calculated from the Nernst equation could not be more negative than -101 mV. We have observed many rods, however, which in 2 mM Cs^+ produce peak potentials significantly more negative than -101 mV. The intercepts of the solid line drawn in Fig. 4 *a* with the 0 mV peak membrane potential and with 2.6 mM $[K^+]_o$ suggest an equilibrium potential close to -93 mV. Such a value would imply an internal K^+ concentration of ~90 mM. The most likely explanation for our observation of peak potentials more negative than E_K is that a component of the current flowing across the rod membrane is electrogenic. Recent experiments involving the use of the cardiac glycoside, strophanthidin, support this view (Owen and Torre, 1981; Torre, 1983).

In Fig. 4 *b* we have plotted the variation in the dark potential, V_D, with external K^+ concentration from data obtained in 136 rods. Note that for concentrations below ~10 mM, the dark potential is only weakly dependent upon $[K^+]_o$. Raising $[K^+]$ above 10 mM, however, produces a substantial depolarization of the rod and for concentrations above ~20 mM, the relation between V_D and log $[K^+]_o$ approaches asymptotically the same line of slope 58 mV that fitted the peak of the light response over that range. This is not surprising because at concentrations >26 mM, a detectable light response could not be elicited and hence the two curves of Fig. 4 coincide at high $[K^+]_o$.

The weak dependence of V_D on $[K^+]_o$ at concentrations below 10 mM requires some comment. Three possible explanations come to mind. First, the K^+ conductance of the membrane might be a function of $[K^+]_o$. The decrease in driving force on K^+ as $[K^+]_o$ is raised would then be offset by an increase in g_K.

Second, raising $[K^+]_o$ may reduce the number of light-modulated channels that are open in darkness (Yau et al., 1981). Thus, at low $[K^+]_o$, g_{Na} might be significantly increased, whereas at high $[K^+]_o$, it could become small enough that the membrane would approach the behavior of a K^+ electrode.

Third, the rate of pumping of an electrogenic Na^+-K^+

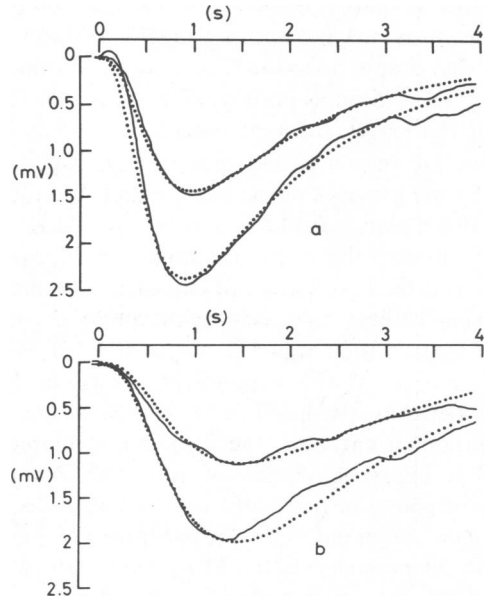

FIGURE 5 Responses to diffuse flashes of light. Stimulus intensities 1.3 Rh*/flash/rod and 2.2 Rh*/flash/rod (*a*) in control Ringer's solution and (*b*) in 10 mM$[K^+]_o$. The dotted lines are theoretical time courses generated as described in the text.

pump would be expected to depend upon $[K^+]_o$. Thus, as $[K^+]_o$ is raised from 2.6 to ~10 mM, the pump should be stimulated so that the decrease in driving force on K^+ would be offset by an increasing electrogenic current. As mentioned earlier, we found evidence that the rod contains an electrogenic mechanism that contributes to its membrane potential at K^+ concentrations in the range tested in Fig. 4. It seems likely, therefore, that this mechanism is at least partly responsible for the weak dependence of the rod's dark potential upon $[K^+]_o$ at concentrations below ~10 mM.

Evidence That in 10 mM $[K^+]_o$, the Effect of the Inductance is Negligible

If in 10 mM $[K^+]_o$ the effect of the inductance were no longer significant, the time course of the voltage response should follow closely that of the photocurrent. This can be checked in two ways. In Fig. 5 *a* the voltage responses elicited in control Ringer's solution have been fitted by the analytical function: $V(t) = A(e^{-\alpha t} - e^{-\beta t})^n$ (Torre and Owen, 1983, Eq. 27). The values of α, β, and n were, respectively, 0.1823 s^{-1}, 3.119 s^{-1}, and 4 for the smaller response. For the larger response, they were 0.1790 s^{-1}, 3.415 s^{-1}, and 4. From Eq. 29 of the preceding paper, using this description of the photovoltage and our standard values of the network parameters, we calculated the time course of the photocurrent in each case. These are shown, appropriately scaled, as the dotted lines in Fig. 5 *b*. The good agreement between the time course of this theoretical photocurrent and of the photovoltages elicited in 10 mM K^+ strongly supports the conclusion that in 10 mM K^+, the effect of the inductance is negligible.

The reverse of this procedure provides a second check. Here we fit Eq. 27 to the photovoltages elicited in 10 mM K^+. This was assumed to provide an analytical description of the photocurrent. By making this equal to $I(\tau)$ in Eq. 15 of the preceding paper, we were able to predict the voltage responses to displaced slits in control Ringer's solution.

Fig. 6 shows a slit experiment performed in 10 mM K^+ and in control Ringer's solution (continuous traces). The dotted lines in Fig. 6 *a* were generated by Eq. 27 with $n = 4$, $\alpha = 0.237\ s^{-1}$, and $\beta = 4.37\ s^{-1}$. They have been scaled to fit the voltage responses. The dotted lines in Fig. 6 *b* are the graphical solutions of Eq. 15 when the current is the analytical function shown in Fig. 6 *a*, and the circuit parameters have the values listed in the figure legend. The values obtained using this procedure in seven different rods are shown in Table II. We shall refer to them as our final estimate of the network parameters.

The average values in control Ringer's solution may be compared with the first estimate and the standard values obtained in the preceding paper. These values are compared in Table III. The good agreement with these independently calculated values provides further support for our conclusion that, in 10 mM K^+, the effect of the inductance is negligible.

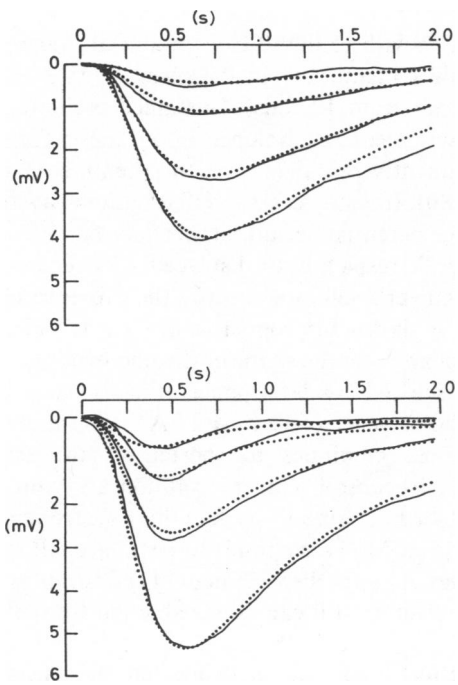

FIGURE 6 Responses to slits of light flashed at distances 0, 10, 20, and 30 μm from the impaled rod (*a*) in the presence of 10 mM$[K^+]_o$ and (*b*) in control Ringer's solution. The circuit parameters have the values R_s = 446 MΩ, g_1 = 0.4 · 10^{-9}S, g_2 = 1.3 · 10^{-9}S, and L = 0.25 · 10^9H. The theoretical curves (dotted lines) were generated as described in the text.

TABLE II
FINAL ESTIMATE OF THE CIRCUIT PARAMETERS: DATA FROM 7 RODS

n	α	β	R_s	g_1	g_2	L
			MΩ	$\times 10^{-9}$S	$\times 10^{-9}$S	$\times 10^9$H
3	0.284	2.721	470	0.125	1.8	0.25
3	0.429	3.09	446	0.4	1.38	0.25
4	0.192	3.0	389	0.29	1.0	0.27
4	0.235	3.051	345	0.33	0.67	1.0
4	0.334	2.12	366	0.1	0.85	0.9
4	0.173	2.82	159	0.25	1.032	0.53
4	0.184	2.7	264	0.236	1.067	0.573
Mean values			361 ± 46	0.3 ± 0.07	1.14 ± 0.2	0.52 ± 0.14

TABLE III
VALUES OF THE CIRCUIT PARAMETER ESTIMATED BY THE THREE INDEPENDENT METHODS DESCRIBED

	First estimate	Standard values	Final estimate
g_1	0.47 × 10^{-9}S	0.26 ± 0.03 × 10^{-9}S	0.3 ± 0.07 × 10^{-9}S
g_2	1.0 × 10^{-9}S	0.88 ± 0.08 × 10^{-9}S	1.4 ± 0.2 × 10^{-9}S
R_s	388 MΩ	310 ± 24 MΩ	361 ± 46 MΩ
L	0.4 × 10^9H	0.74 ± 0.1 × 10 s	0.52 ± 0.14 × 10^9H

Values of the Circuit Parameters When $[K^+]_o$ = 10 mM

Using double-barreled electrodes (see preceding paper), we measured the network input impedance both in control Ringer's solution and in Ringer's solution containing 10 mM K^+. On raising $[K^+]_o$ to 10 mM, there was a 12% decrease in the steady-state input impedance; i.e., from 108 to 94 MΩ (average values from nine cells). In 10 mM $[K^+]_o$, the steady-state length constant was 18 μm. Using these values, we calculate that in 10 mM $[K^+]_o$, R_s = 264 MΩ and $(g_1 + g_2) = 1.2 \times 10^{-9}$S. Taking the instantaneous value of the length constant (see preceding paper) to be 20 μm, we obtain the circuit parameters in 10 mM $[K^+]_o$ Ringer's solution: $g_1 = 0.95 \times 10^{-9}$S, $g_2 = 0.25 \times 10^{-9}$S, R_s = 264 MΩ, and $L = 2.6 \times 10^9$H.

The increase in L is necessary to account for the negligible change in the time constant and has no other significance. These values indicate that the fraction of total membrane current flowing through the arm of the circuit containing the inductance is reduced by a factor of 4 when $[K^+]_o$ is raised from 2.6 to 10 mM.

Circuit Parameters and the Flash Sensitivity of the Rod

The flash sensitivity was determined from linear range responses to weak, diffuse flashes of light. Typical data are shown in Fig. 5. The continuous traces are responses elicited by flashes that produced, on average, 1.3 Rh*/flash and 2.2 Rh*/flash. In control Ringer's solution (Fig. 5 *a*), the times-to-peak of both responses were ~1 s. Raising $[K^+]_o$ to 10 mM (Fig. 5 *b*) caused a depolarization in darkness of 1.5 mV and an increase in time-to-peak for the responses to ~1.5 s, consistent with a reduced effect of the inductance in the equivalent circuit. The flash sensitivity of this rod decreased from 1.16 mV/Rh* in control Ringer's solution to 0.9 mV/Rh* in 10 mM $[K^+]_o$.

The collected data from 27 rods are presented in Table IV. Note that raising $[K^+]_o$ from 2.6 to 5 mM had only a small effect on either dark potential or flash sensitivity, whereas at concentrations above 10 mM both dark potential and flash sensitivity were reduced significantly. On average, raising $[K^+]_o$ to 10 mM produced a 35% decrease in flash sensitivity. In experiments in which measurements were made with slit illumination, a decrease of ~50% was recorded. The change in circuit parameters accounts only for a 20% decrease in flash sensitivity measured with diffuse illumination and an 18% decrease with slit illumination (calculations made using the theoretical photocurrent of the preceding paper). This discrepancy may be accounted for by supposing that, in addition, external K^+ acts directly to reduce the magnitude of the photocurrent, an action for which there is new evidence (Yau et al., 1981).

TABLE IV
EFFECTS OF VARYING $[K^+]_o$ UPON THE ROD'S DARK POTENTIAL AND FLASH SENSITIVITY: SUMMARY OF DATA FROM 27 RODS

Number of cells	Flash sensitivity in control Ringer's solution	$[K^+]_o$	ΔV_m	Flash sensitivity in test solution
	*mV/Rh**	*mM*	*mV*	*mV/Rh**
4	1.02 ± 0.27	3.5	0	1.19 ± 0.56
2	1.6 ± 0.3	4.5	0	1.7 ± 0.1
2	0.73 ± 0.2	5	0	0.58 ± 0.1
7	1.0 ± 0.06	9	1.6 ± 0.3	0.7 ± 0.1
5	0.96 ± 0.13	10	1.62 ± 0.35	0.6 ± 0.06
4	0.85 ± 0.06	14	6.25 ± 0.9	0.29 ± 0.03
3	0.73 ± 0.04	19	9.66 ± 1.6	0.27 ± 0.03

Effect of TEA on High-Pass Filtering

It is well known that TEA^+ ions are specific blockers of voltage- and time-dependent K^+ channels in a variety of preparations, though the mechanism of the blocking action may vary from preparation to preparation (Armstrong and Binstock, 1965; Armstrong, 1966, 1969, 1971; Hille, 1967; Armstrong and Hille, 1972). We perfused the toad retina with Ringer's solutions to which had been added concentrations of TEA^+ from 5 to 20 mM. In another paper (Torre and Owen, 1983) we present a more detailed analysis of the effects of TEA^+ on the rod membrane and on the rod's voltage response to light seen in these experiments.

Perfusion with 15 mM TEA^+ produced a depolarization of the rod's membrane in darkness of 10.2 ± 1.1 mV (SEM, data from 12 rods). Sustained oscillations of the membrane potential developed in darkness; these oscillations frequently resembled action potentials (Fain et al., 1977, 1980). In many cases, oscillations died away and the membrane potential became stable once more.

In Fig. 7, responses to displaced slits recorded in (*a*) control Ringer's solution and (*b*) the presence of 15 mM TEA^+ are shown for comparison. Clearly, TEA caused striking changes in the shape of the photovoltage and these will be discussed in a later paper. It is also clear, however, that in the presence of 15 mM TEA^+, the time-to-peak of the response continues to shorten as the stimulus is displaced. In control Ringer's solution a 30-μm displacement reduced the time-to-peak in this case from 580 to 430 ms. In 15 mM TEA, times-to-peak are all somewhat longer, but a 40-μm displacement of the stimulus from the centered position still caused a reduction from 890 to 690 ms.

The effect upon the network length constant, λ, is illustrated in Fig. 7 *c*. In this particular cell, λ fell from 18 to ~12 μm during the first 2 s of the response in control Ringer's solution. Adding 15 mM TEA caused the initial value of λ to increase to ~43 μm, but this fell rapidly to ~10

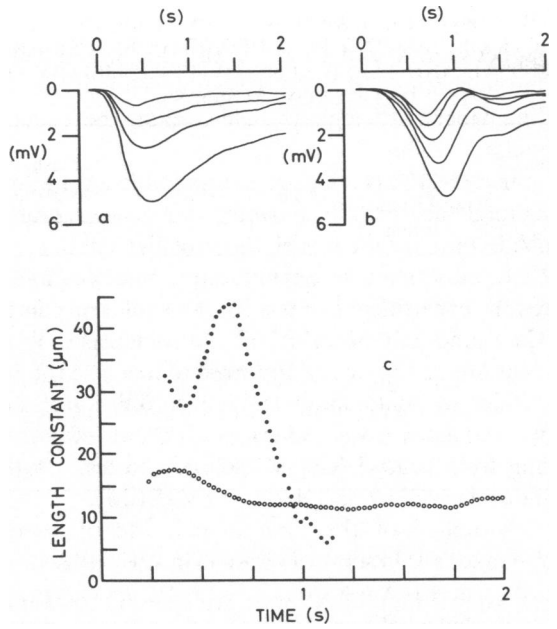

FIGURE 7 The effect of 15 mM TEA^+ on high-pass filtering. Responses to displaced slits recorded (*a*) in control Ringer's solution, and (*b*) in Ringer's solution containing 15 mM TEA^+. (*c*) The network length constant is plotted as a function of time from the records shown in *a* and *b*.

μm within the first 0.5 s of the response. These observations are all consistent with the notion that the inductance-like mechanism is not blocked by TEA, at least when applied extracellularly. An alternative explanation must also be considered, however.

The observation that TEA induces sustained oscillations of the membrane potential and even spike-like potentials implies that, in the presence of TEA, the membrane contains a voltage-dependent, time-varying conductance that, for small perturbations of voltage, should mimic the behavior of an inductance. Our evidence indicates that this oscillatory behavior is primarily due to a Ca^{++} conductance that becomes regenerative at potentials positive with respect to the rod's normal dark potential (Torre and Owen, 1983; Bader, et al., 1982; Fain et al., 1978). Although inductancelike behavior can result from a K^+ conductance activated by membrane depolarization, it could equally well be due to, for small perturbations, the inactivation of such a Ca^{++} conductance. Thus, TEA might block the voltage-dependent $g_K(=g_2)$ but, perhaps as a result of the concomitant depolarization, reveal a second Ca^{++}-dependent mechanism that mimics an inductance.

The observation that quite low concentrations of Co^{++} block the regenerative behavior seen in TEA (Fain et al., 1978; Torre and Owen, 1982; Bader, et al., 1982) provides a test of this interpretation. We performed the slit experiment while perfusing with 15 mM TEA and concentrations of Co^{++} up to 1 mM. With Co^{++} added to the medium, the marked depolarization of the rod seen in TEA alone no longer occurred. Despite this and the absence of regenerative behavior, the high-pass filtering by the network remained and was more marked than in control Ringer's solution.

We noted earlier that perfusion with 10 mM K^+ was sufficient to abolish high-pass filtering. When TEA was added to a perfusate containing 10 mM K^+, high-pass filtering reappeared. A 40-μm displacement of the slit caused a reduction in the time-to-peak of the response from 740 to 605 ms in the presence of 10 mM K^+ and 15 mM TEA, whereas in 10 mM K^+ alone, no such reduction occurred. Raising the external $[K^+]$ to 20 mM was sufficient to eliminate the high-pass filtering in the presence of 15 mM TEA.

These results are most easily explained if TEA blocks an ionic conductance that plays no part in the mechanism of high-pass filtering. In terms of the equivalent circuit model, such a conductance will contribute to g_1. Of course, whether or not high-pass filtering is observed will depend upon the relative magnitudes of the currents flowing through g_1 and g_2. 10 mM K^+ may reduce the current through g_2 sufficiently, under normal conditions, to eliminate high-pass filtering. The reduction of g_1 by TEA, however, may redistribute the current such that the circuit again behaves as a high-pass filter and further reduction of the driving force on K^+ will be required to eliminate this behavior once more.

In summary, the consistently observed increase in λ_{in} caused by extracellularly applied TEA implies that it acts to block ionic channels in the plasma membrane. Moreover, the concomitant depolarization of the membrane in darkness is consistent with a blocking of K^+ channels by TEA. Other results described in this section, however, indicate that if this interpretation is correct, the K^+ channels blocked by TEA are a different population from those responsible for the high-pass filtering behavior of the network.

Effects of Reducing External $[Ca^{++}]$

It is possible that the voltage-dependence of the K^+ conductance responsible for high-pass filtering is a secondary characteristic. If the K^+ conductance were dependent upon internal $[Ca^{++}]$, it would merely reflect voltage-dependent changes in $[Ca^{++}]_{in}$. K^+ conductances that behave in this way have been described in many preparations (reviews by Baker, 1972; Reuter, 1973; Meech, 1978; also see Hagiwara, 1973; Meech, 1974; Meech and Standen, 1974, 1975; Beaty and Stefani, 1976; Thompson, 1977; Atwater et al., 1979). While in many preparations such conductances can be blocked by externally applied TEA, this is not the case in others (Thompson, 1977; Beaty and Stefani, 1976).

Many experiments were performed in Ringer's solutions containing nominally zero Ca^{++}. Measurements of residual Ca^{++} concentrations in these solutions, generously

performed by Dr. T. Rink and Dr. R. Tsien using a Ca^{++}-specific electrode of their own design, yielded values between 20 and 40 μm. Solutions referred to hereon as 0 Ca^{++} Ringer's solution, therefore, contained ~30 μM of residual Ca^{++}.

Perfusion with 0 Ca^{++} Ringer's solution caused the rod membrane to depolarize by 27.2 ± 4.2 mv (SEM, eight cells). The increase in amplitude of the response to a saturating, diffuse flash was equivalent to this depolarization, as reported earlier by Brown and Pinto (1974). In Fig. 8 we present data from two slit experiments performed (*a*) in control Ringer's solution and (*b*) in 0 Ca^{++} Ringer's solution. Stimuli produced, on average, 28.7 Rh*/flash. In control Ringer's solution, the time-to-peak decreased, in this case, from 870 to 500 ms as the stimulus was displaced laterally by 37.5 μm. On switching to 0 Ca^{++} Ringer's solution, the time-to-peak of the response to the centered stimulus almost doubled to ~1,500 ms and did not change as the stimulus was displaced. In Fig. 8 *c* it can be seen that in control Ringer's solution λ fell from 27 to 14 μm during the response, while in 0 Ca^{++} Ringer's solution it remained at a low value of 9–11 μm, indicating that high-pass filtering had been eliminated.

There are several possible interpretations of these results. The very low value of λ_{in} in 0 Ca^{++} Ringer's solution implies a large increase in g_1 of the model. In similar experiments on eight different rods, the value of λ_{in} fell from an average 28 μm to an average 14 μm (Table I). From our analysis of the equivalent circuit model, assuming no large change in R_s, we estimate that g_1 increased by a factor of about 4, although since, in control Ringer's solution, measured values of λ_{in} are almost certainly smaller than the true instantaneous value (limiting value as $t \rightarrow 0$); this factor may actually be somewhat larger, perhaps between 4 and 6. By itself, this would not be sufficient to eliminate the high-pass filtering behavior (see preceding paper), although it would certainly be a contributing factor.

Of course, the K^+-conductance responsible for high-pass filtering might also be fully activated by a depolarization of ~30 mV. In terms of the model, this is equivalent to $g_2 \rightarrow 0$ (Eq. 2). In an attempt to eliminate this complication, we repeated the experiment in a test Ringer's solution containing 0 Ca^{++} and only 50 mM Na^+. In some cases, 6 mM Mg^{++} was added because it appeared to increase the time during which we could obtain stable recordings in this test solution, though it was otherwise without effect. On switching from control Ringer's solution to test solution, the rod depolarized by only 4.2 ± 1 mV (SEM).

The shortening of the time-to-peak, clearly seen in control Ringer's solution, was absent in the test solution. Moreover, whereas λ fell from ~33 to ~12 μm during the response in control Ringer's solution, it remained between 12 and 16 μm in the test solution. This suggests that the elimination of high-pass filtering in the presence of 0 Ca^{++} is not directly attributable to a membrane potential change, but is brought about primarily by the reduction in the external concentration of Ca^{++}. The reason for this became clear upon comparing responses to weak, diffuse flashes of light recorded in control Ringer's solution and in a 0 Ca^{++} test solution.

Supralinearity in 0 Ca^{++}

Responses elicited by diffuse stimuli in control Ringer's solution and in 0 Ca^{++} Ringer's solution are shown in Figs. 9 *a* and *b*, respectively. Four stimulus intensities were used,

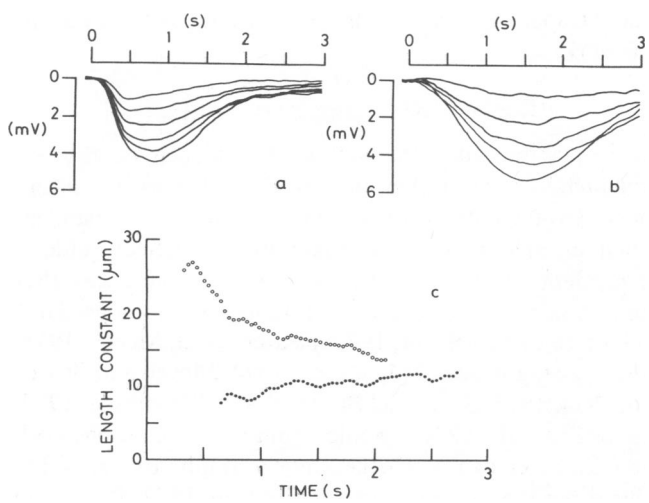

FIGURE 8 The effect of reducing $[Ca^{++}]_o$ to 30 μM on high-pass filtering. Responses to displaced slits recorded (*a*) in control Ringer's solution, and (*b*) in 0 Ca^{++} Ringer's solution ($[Ca^{++}]_o \approx 30$ μM). (*c*) The network length constant is plotted as a function of time from the records shown in *a* and *b*. ●, 30 μM Ca^{++}; O, normal Ringer's solution.

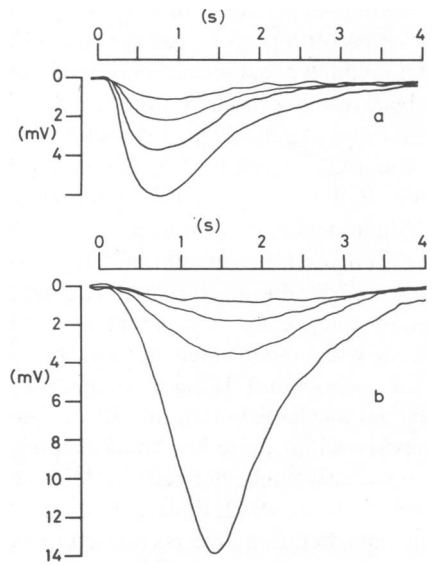

FIGURE 9 Responses to diffuse stimuli of intensities 1.3, 3.2, 5.86, and 14.06 Rh*/flash/rod as recorded (*a*) in control Ringer's solution, and (*b*) in 0 Ca^{++} Ringer's solution ($[Ca^{++}]_o \approx 30$ μM).

which produced on average 1.3, 3.2, 5.86, and 14.06 Rh*/flash. The time-to-peak of the response to a given stimulus in the 0 Ca^{++} Ringer's solution was again nearly double that in control Ringer's solution. More significantly, perhaps, the responses in the test solution did not show a linear relation between peak amplitude and stimulus intensity. The response to 14.06 Rh*/flash is four times larger at peak than that to 5.86 Rh*/flash. The relation between peak photovoltage and stimulus intensity is thus supralinear. As a consequence of this, it is not possible to define a unique flash sensitivity (millivolts per photoisomerization) of the rod in 0 Ca^{++} Ringer's solution.

Supralinear behavior of this type could be produced by a regenerative ionic current, though it is unlikely that such a current could be carried by the small number of residual Ca^{++} ions in the external medium. Significantly, Yau, et al., (1981) observed a similar supralinearity in 0 Ca^{++} Ringer's solution, recording the photocurrent across the outer segment membrane of the toad rod.

Lamb et al. (1981) noted that the relation between the photocurrent, J, and the stimulus intensity, I, *at a fixed time after stimulation* was not well described by a Michaelis-Menten equation, but was better fitted by a function of the form

$$J/J_{max} = 1 - e^{-I}. \tag{3}$$

This relation is steeper than a Michaelis-Menten relation plotted on the same coordinates, though it is not supralinear.

Of course, we have measured photovoltages in the present study. Provided no voltage- and time-dependent conductance changes occur between stimulation and the time at which V is measured, however, we can assume that the membrane potential in darkness is determined simply by

$$V = \frac{\tilde{g}E}{\bar{g} + \tilde{g}}, \tag{4}$$

where E is a battery in series with a fixed conductance, $\tilde{g}$, and $\bar{g}$ is the light-sensitive conductance (Baylor et al., 1974). If we make the further assumption that the fraction of light sensitive channels $\Delta g/g$ blocked by the light intensity I is

$$\Delta\bar{g}/\bar{g} = 1 - e^{-I} \tag{5}$$

instead of a Michaelis-Menten relation, then from Eqs. 3–5 we obtain

$$\frac{\Delta V}{\Delta V_{max}} = \frac{1 - e^{-I}}{1 + Ke^{-I}}, \text{ where } K = \frac{\bar{g}}{\tilde{g}}. \tag{6}$$

K is thus the ratio of the total light-sensitive conductance, $\bar{g}$, to the fixed shunting conductance, $\tilde{g}$. Eq. 6 exhibits supralinear behavior provided $K \gtrsim 3$.

A series of responses to diffuse stimuli of increasing intensities was recorded in 0 Ca^{++} Ringer's solution. Responses are shown on two different time scales in Figs. 10 *a* and *b*. In Fig. 10 *c*, we have plotted the ratio ΔV:ΔV_{max}

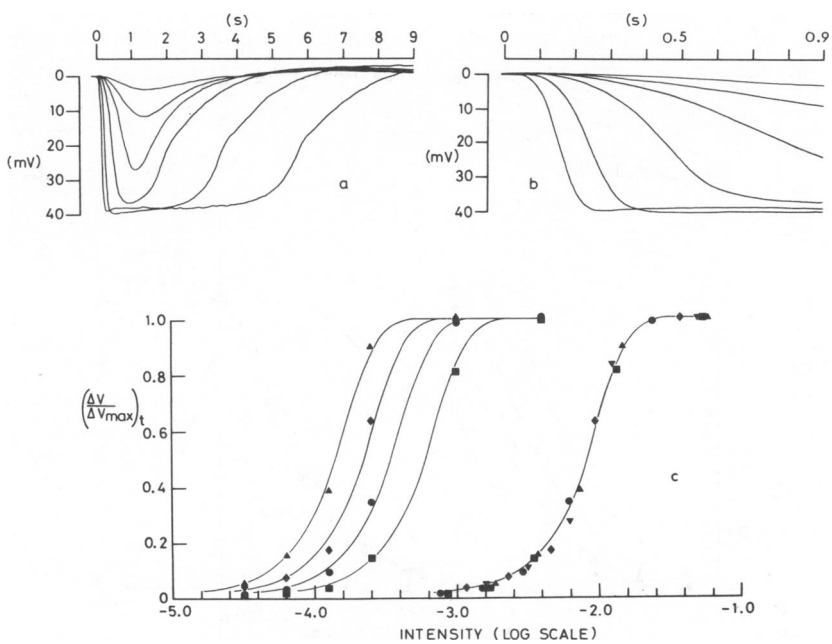

FIGURE 10 Responses to a series of diffuse stimuli of increasing intensity recorded in 0 Ca^{++} Ringer's solution ($[Ca^{++}]_o \approx 30\ \mu M$) (*a*). (*b*) Records are shown on two different timescales. (*c*) The ratio ΔV:ΔV_{max} measured at fixed times between 300 and 700 ms after stimulus onset is plotted as a function of stimulus intensity. The right-hand curve was obtained by arbitrarily translating the sets of data along the abscissa so that they superimposed. ■, 300 ms; ●, 400 ms; ♦, 500 ms; ▼, 600 ms; and ▲, 700 ms. The solid curve drawn through each of the sets of data points is a plot of Eq. 6 with $K = 10$.

at fixed times from 300 to 700 ms after stimulation. Arbitrarily translating each set of data points along the abscissa allows them to be superimposed (right-hand curve). The fact that all data then lie on a single curve argues that no time-varying, voltage-dependent conductances were activated within the interval 300–700 ms. We therefore fitted Eq. 6 to the data. A best fit was obtained with $K = 10$.

Because time-varying, voltage-dependent conductances clearly contributed to responses in control Ringer's solution over the same time interval, we were unable to determine a reliable value of K in that case. Given that, in control Ringer's solution, the rod's dark potential lies about halfway between E_K and E_{Na}, however, we estimate that $\bar{g}$ and $\tilde{g}$ must be nearly equal (i.e., to a first approximation $K = 1$). Provided that $\tilde{g}$ does not change significantly, the increase in the value of K on switching to 0 Ca^{++} Ringer's solution implies a roughly fivefold increase in total membrane conductance. This agrees well with the factor of 4–6 estimated earlier on the basis of slit experiments in 0 Ca^{++} Ringer's solution. Thus, it is possible to explain the effects of 0 Ca^{++} Ringer's solution on the network length constant and the appearance of supralinear behavior in terms of a 10-fold increase in the magnitude of the light sensitive conductance.

If this explanation is correct, any ionic manipulation that either decreases $\bar{g}$ or increases $\tilde{g}$ should decrease or abolish the supralinear behavior. We therefore tested the hypothesis in the following way. It was noted earlier that increasing $[K^+]_o$ to 20 mM or more caused the rod's membrane to behave, in darkness, in a manner similar to a K^+ electrode (Fig. 4 *b*). This implies that the ratio of the light-sensitive conductance to the K^+ conductance is decreased substantially below its value in control Ringer's solution. We therefore repeated the experiment of Fig. 10 in a 0 Ca^{++} Ringer's solution containing 26 mM K^+. The results are shown in Fig. 11. Examination of the responses (shown in Figs. 11 *a* and *b* on two different time scales) reveals no evidence of supralinear behavior. In Fig. 11 *c* the ratio $\Delta V{:}\Delta V_{max}$, measured at fixed times between 300 and 800 ms after stimulation is plotted. Once again, all the data can be fitted by Eq. 6, this time with $K = 2$.

Effect of Co^{++}

In other systems it has been shown that Ca^{++}-dependent K^+ conductances can often be blocked by adding low concentrations of Co^{++} to the bathing medium (Hagiwara, 1973; Meech and Standen, 1975). We therefore performed a series of experiments in which the test Ringer's solution contained Co^{++} in concentrations of between 50 and 1,000 μM. In darkness the rod membrane hyperpolarized by 4–12 mV upon exposure to concentrations of Co^{++} between 50 μM and 1 mM, and the time course of the photovoltage became slower. This can be seen in Fig. 12, which illustrates a typical slit experiment performed (*a*) in control Ringer's solution and (*b*) in the presence of 1 mM Co^{++}. This particular cell hyperpolarized rapidly in darkness by 7 mV and then slowly repolarized by 2 mV during

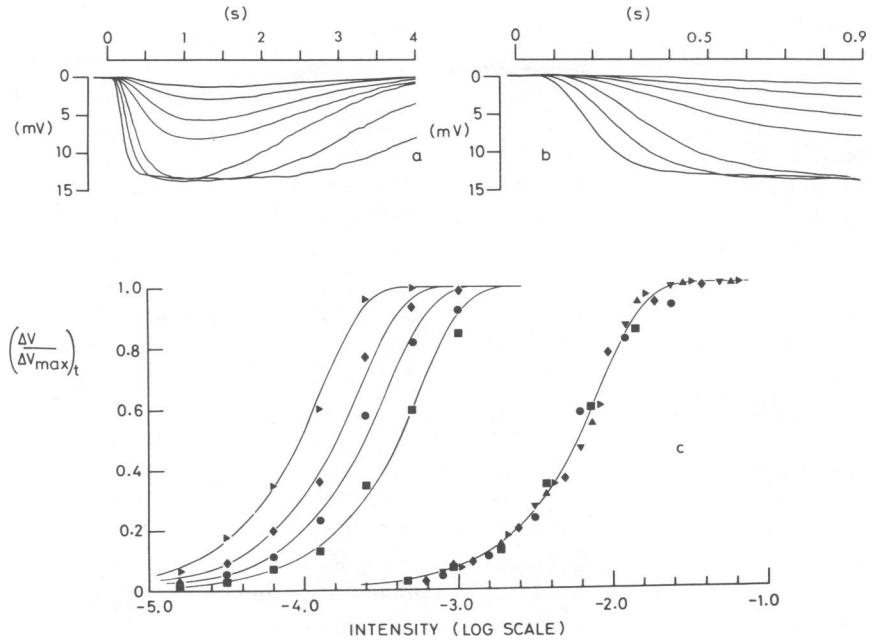

FIGURE 11 Responses to a series of diffuse stimuli of increasing intensity recorded in Ringer's solution containing 30 μM Ca^{++} and 26 mM K^+ (*a*). (*b*) Records are shown on two different timescales. (*c*) The ratio $\Delta V{:}\Delta V_{max}$ measured at fixed times between 300 and 800 ms after the onset of the stimulus is plotted as a function of stimulus intensity. The right-hand curve was obtained by arbitrarily translating each individual set of data until they superimposed. ■, 300 ms; ●, 400 ms; ♦, 500 ms; ▼, 600 ms; ▲, 700 ms; and ▶, 800 ms. The solid curve drawn through each of the sets of data is a plot of Eq. 6 with $K = 2$.

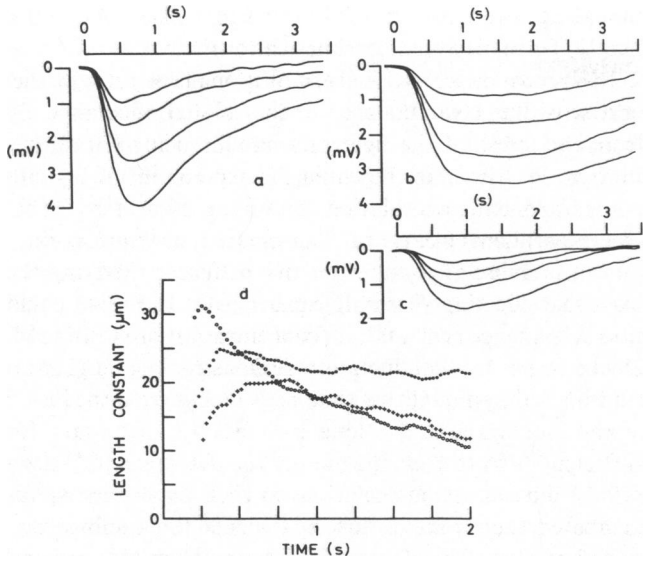

FIGURE 12 Effect of 1 mM Co^{++} on high-pass filtering. Responses to displaced slits of light recorded (*a*) in control Ringer's solution, (*b*) in Ringer's solution containing 1 mM Co^{++}, and (*c*) in control Ringer's solution containing 1 mM Co^{++} during exposure to a background of intensity 4.3 Rh*/s. (*d*) The network length constant is plotted as a function of time from the records shown in *a*, *b*, and *c*. ●, 1 mM Co^{++}; +, 1 mM Co^{++} with background; O, normal Ringer's solution.

the 3 min after exposure to Co^{++}. Significantly, the shortening of the time-to-peak seen in control Ringer's solution when stimuli were displaced was virtually eliminated in the presence of Co^{++}. In Fig. 12 *d* it can be seen that, whereas the network length constant fell from ~32 to ~12 μm during the response in control Ringer's solution, when Co^{++} was added the fall in λ was much less marked, from 24 to 21 μm over the same interval. In 21 such experiments, λ fell from an average 25.8 to 13.9 μm in control Ringer's solution, whereas in the presence of Co^{++}, the corresponding values were 20.6 and 16.8 μm. Clearly the addition of Co^{++} in concentrations as low as 200 μm substantially reduced the degree of high-pass filtering by the rod network.

When we repeated the experiment in the presence of Co^{++} against a steady background illumination that produced, on average, 4.3 Rh*/s in each rod, the high-pass filtering reappeared (Fig. 12 *c*). The time-to-peak of each response was shorter than that recorded in the absence of background illumination and again showed the familiar dependence on stimulus position. As seen in Fig. 12 *d*, the fall in λ during the response closely matched that seen in control Ringer's solution at times later than ~600 ms after stimulus onset.

The increase in λ_{ss} is consistent with a reduction in g_l by Co^{++}. The reduction in high-pass filtering is accounted for by the slowing of the response (Torre and Owen, 1983, Fig. 12). This explanation also accounts for the reemergence of high-pass filtering in the presence of a background that shortened the response time course once more.

The high-pass filtering behavior was similarly restored in the presence of Co^{++} when 15 mM TEA was added to the test Ringer's solution. In those experiments, the effect of TEA was to repolarize the rod membrane approximately to the dark potential in control Ringer's solution. In view of these findings we conclude that if the K^+ conductance responsible for high-pass filtering is Ca^{++}-dependent, it is not significantly affected by application of Co^{++} or of TEA.

The persistence of high-pass filtering in the presence of Co^{++} argues strongly against a mechanism dependent upon chemical synapses. This interpretation receives further support from experiments in which Na aspartate or Na glutamate were added in concentrations sufficient to saturate horizontal cells yet which had no discernible effect upon the high-pass filtering. Concentrations of Mg^{++} as high as 6 mM were also without effect. A summary of all the results is given in Table I.

DISCUSSION

In the previous paper, three possible models were proposed to explain the high-pass filtering of small signals by the rod network (Torre and Owen, 1983, Fig. 2). Model 1 postulated the existence in the rod membrane of a time-varying, voltage-dependent conductance that, for small voltage changes, mimics the behavior of an inductance. Model 2 supposed the existence of an equivalent capacitance between neighboring rods in parallel with the shunting conductance. Model 3 attributed the high-pass filtering to electrical and chemically mediated interactions between rods, occurring in parallel.

A prediction of Model 2 is that any ionic manipulation that abolishes high-pass filtering without affecting the time course of the photocurrent should have no effect upon the time course of the voltage response to a diffuse flash of light. Experiments with high $[K^+]_o$ showed that this prediction was not fulfilled (Fig. 5). Furthermore, the persistence of high-pass filtering in the presence of Co^{++} at concentrations sufficient to block synaptic transmission between receptors and second-order cells (Kaneko & Shimazaki, 1975), and in the presence of Na aspartate, Na glutamate, and Mg^{++} argues against Model 3. By contrast, all the major results can be described both qualitatively and quantitatively in terms of Model 1, and we feel justified, therefore, in ascribing the high-pass filtering behavior of the rod network to time- and voltage-dependent properties of the rod membrane.

It is possible to imagine that high-pass filtering might reflect some time-varying property of the light-dependent channels themselves. Such a possibility can be discounted, however, because experiments with suction pipettes have shown that the current flowing across the outer segment membrane of the rod does not change significantly when neighboring rods are illuminated (Baylor et al., 1979). Thus, any time-dependent properties of the light-modu-

lated channel can be of no significance for high-pass filtering, which is a property of the rod network.

Experiments in which $[K^+]_o$ was raised to 10 mM or more indicated that the current flowing through the arm of the circuit containing the inductance is primarily, if not exclusively, carried by K^+. Although increasing $[K^+]_o$ from 2.6 to 10 mM depolarized the rod by <2 mV, the amplitude of the light response was markedly diminished. One reason for this might be that $[K^+]_o$ is capable either of blocking the light-modulated conductance directly or of reducing the Na^+ current by some sort of competitive inhibition. Recent measurements of photocurrents made by the suction pipette technique have revealed a 30–50% reduction in the magnitude of the photocurrent upon increasing $[K^+]_o$ from 2.6 to 10 mM and even larger decreases with higher concentrations of K^+ (Yau et al., 1981). This effect of $[K^+]_o$ would contribute to the reasons why at concentrations of K^+ above 10 mM, the behavior of the rod membrane approached that of a K^+ electrode.

The possibility remains that the K^+ conductance responsible for high-pass filtering is gated by internal Ca^{++} and thus not directly voltage-dependent. Our attempts to examine this possibility were inconclusive because of the somewhat complicated effects of changing $[Ca^{++}]_o$ and of adding Co^{++} to the bathing medium.

The primary effect of reducing $[Ca^{++}]_o$ was to increase the magnitude of the light-sensitive conductance. The effects of 0 Ca^{++} Ringer's solution on the network length constant and the appearance of supralinear behavior could be explained in terms of a 10-fold increase in the light-sensitive conductance relative to its magnitude in control Ringer's solution.

The effects of Co^{++} were also complicated. Concentrations of $[Co^{++}]_o$ as low as 50 μM hyperpolarized the membrane by 5–7 mV, caused a marked increase in the time-to-peak of voltage responses to dim flashes of light, and prolonged the subsequent repolarization to the dark potential. The return to the dark potential after exposure to bright, diffuse flashes was also substantially prolonged.

Four divalent cations, Co^{++}, Ca^{++}, Sr^{++}, and Mg^{++} were found to hyperpolarize the membrane in darkness. Co^{++} was 10–100 times as effective as Ca^{++} which, in turn, was 2–5 times as effective as Sr^{++} or Mg^{++}. One interpretation of this finding is that divalent cations may block membrane conductances by changing the density of surface charge. A second possibility is that they may displace the range of activation of voltage-dependent conductances, thereby causing a change in membrane conductance and a hyperpolarization of the cell. Yet another possibility that must be considered is that they might all act directly to block the light-sensitive conductance, though with different affinities for the blocking site.

Analysis of the effect of Co^{++} upon the time course of the voltage response suggests that the prolongation we observe probably results from a slowing of the decay phase of the photocurrent. A similar effect is seen with $Mn++$, though at lower concentrations (M. Capovilla, L. Cervetto, and V. Torre, personal communication).

In a recent paper, Attwell and Wilson (1980) studied the current-voltage relations of rods isolated mechanically from the retina of the tiger salamander. They found that they could dissociate the voltage-gated conductances into two components, one blocked by Cs^+, the other by TEA. Our experiments clearly rule out the Cs^+-sensitive conductance as having any role in the mechanism underlying the high-pass filtering of small signals. Moreover, we could find no evidence that TEA, in concentrations up to 15 mM, blocked the K^+ conductance responsible for high-pass filtering. One possibility is that TEA blocks from the inside of the membrane and a long incubation is necessary for sufficient TEA to cross the membrane. Attwell and Wilson (1980) did not change solutions in their experiments, but incubated their rods in different media for a substantial period of time. The effects of TEA are discussed in detail elsewhere (Torre and Owen, 1983).

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