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THE SODIUM CURRENT UNDERLYING THE RESPONSES OF TOAD RODS TO LIGHT

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SUMMARY

1. Intracellular responses were recorded from single rods in the retina of the toads *Bufo bufo* and *Bufo marinus* during exposure to solutions in which sodium was replaced by equimolar amounts of choline.

2. Upon moderate reduction (80 and 50 mM) of the external sodium the size of responses to bright flashes decreased as a consequence of both an increase in the resting potential and a decrease of the membrane potential at the peak, while the level of the plateau remained fairly constant.

3. Upon reduction of the external sodium to 22 mM or less, rods hyperpolarized to about the plateau level and failed to respond to illumination. Under these circumstances, membrane depolarization induced by an increased external potassium did not restore the cell responsiveness. Addition of 2–5 mM caesium hyperpolarized the membrane and partially restored the photoresponse.

4. Complete replacements of external sodium with potassium depolarized the rod by 40 ± 10 mV, and no voltage responses to light could be detected.

5. In the presence of caesium, a nearly complete blockage of the photoresponses was obtained when the external sodium was 5 mM or less. Further reductions of the external sodium did not invert the photoresponses. Application of caesium when the external sodium was nominally zero induced a transient hyperpolarization followed by a slow decay.

6. During exposure to steady illumination, the dependence of the plateau level on the external sodium slowly increased.

7. These results indicate that the ionic current which is directly modulated by the light depends primarily on the external sodium. They suggest also that the current associated with the voltage- and time-dependent process responsible for the sag from peak to plateau of the response to a bright flash of light may have multiple components.

INTRODUCTION

At present there is a certain amount of evidence to show that the voltage response to light of vertebrate photoreceptors arises from a decrease in the ionic permeability of the outer segments to sodium (Sillman, Ito & Tomita, 1969; Hagins, Penn & Yoshikami, 1970; Korenbrot & Cone, 1972; Cavaggioni, Sorbi & Turini, 1973;

Cervetto, 1973; Brown & Pinto, 1974). The properties of the membrane current of rod outer segments and its dependence on light have been analysed recently in detail by Baylor, Lamb & Yau (1979). Recently it has been suggested that potential- and time-dependent processes contribute to rods' voltage responses to bright flashes of light (Fain, Quandt, Bastian & Gerschenfeld, 1978; Detwiler, Hodgkin & McNaughton, 1980). Results from a voltage-clamp study on isolated rods support this view and show that an inward current slowly develops after a hyperpolarizing step (Bader, MacLeish & Schwartz, 1979).

A previous paper (Capovilla, Cervetto & Torre, 1980) described the influences of potassium and chloride on rod photoresponses and suggested that potential-and time-dependent conductances affect the shape of voltage responses to dim flashes of light. Here, we describe the influence of the external sodium on the intracellular voltage responses of rods. The results support the notion that the photoresponses of vertebrate rods are initiated by a reduction in the sodium current and provide information on the properties of potential- and time-dependent conductances.

Some of these results were presented earlier in summary form at a meeting of the Physiological Society (Capovilla, Cervetto & Torre, 1979).

METHODS

Experiments were performed on the isolated perfused retina of *Bufo bufo* and *Bufo marinus*. The species from which responses were obtained is specified in the caption of the Figures. The retina with receptor side up was maintained at room temperature (21 °C) in a chamber through which an oxygenated and buffered solution flowed at a rate of 2 ml./min. All solutions were freshly prepared; their composition is given in Table 1.

Complete substitution of the medium that bathed the retina required about 10 sec as checked by dyes. Estimates of the rate of changes in the internal concentration upon substitution of the external fluid (see Capovilla *et al.* 1980) indicated that significant changes of the internal sodium concentration are likely to occur when the retina is exposed for more than 1–2 min to a solution containing less than 10 mM-sodium.

Intracellular recordings were made with glass micro-electrodes filled with 3 M-K-acetate and 0·2 M-KCl; their resistance measured in the bathing fluid was 200–600 MΩ. The indifferent electrode was connected to the bath by means of a saturated agar-KCl bridge to minimize changes in junction potentials.

Additional information on the methods for intracellular recording from single rods in the isolated perfused retina of toad and on the techniques used for light and current stimulation have been reported previously (Cervetto, Pasino & Torre, 1977; Capovilla *et al.* 1980).

RESULTS

Substitution of external sodium by choline increased the rod transmembrane potential and reduced the size of photoresponses. The effects induced by reductions of the external sodium up to about half the physiological value promptly reversed upon washing provided the low sodium conditions were maintained for less than 2–3 min. Complete reversibility was also obtained with prolonged exposures and with very low values of the external Na^+, but required periods of washing up to 10 min. Data collected from eighty rods are tabulated in Table 2. A representative example of the effects induced by three different concentrations of external sodium on the resting potential and photoresponses of a rod is illustrated in Fig. 1. It is seen that for progressively lower $[Na^+]_o$ (50, 22 and 10 mM, solutions C, E and G respectively) the

TABLE 1. Ionic composition of the solutions used in the present study. The pH was adjusted at 7·6±0·3 for all solutions

Solutions (mM)...	Na^+	K^+	Choline	Cl^-	HCO_3^-	Ca^{2+}	Mg^{2+}	Glucose	Tris	Cs^+	Co^{2+}	TEA
A (control)	132	2·6	—	117·4	22	1·6	0·8	5	—	—	—	—
B	80	2·6	50	115·4	22	1·6	0·8	5	—	—	—	—
C	50	2·6	80	115·4	22	1·6	0·8	5	—	—	—	—
D	32	2·6	100	117·4	22	1·6	0·8	5	—	—	—	—
E	22	2·6	110	117·4	22	1·6	0·8	5	—	—	—	—
F	15	2·6	110	117·4	15	1·6	0·8	5	—	—	—	—
G	10	2·6	110	120·8	10	1·6	0·8	5	5	—	—	—
H	5	2·6	110	124·1	5	1·6	0·8	5	10	—	—	—
I	0	2·6	110	124·1	0	1·6	0·8	5	10	—	—	—
J	22	10	102·6	117·4	22	1·6	0·8	5	—	—	—	—
K	22	26	86·6	117·4	22	1·6	0·8	5	—	—	—	—
L	40	90	4·6	117·4	22	1·6	0·8	5	—	—	—	—
M	0	110	—	121·5	—	1·6	0·8	5	10	—	—	—
N	0	100	22	111·8	22	1·6	0·8	5	—	5	1	10
O	22	2·6	110	119·4	22	1·6	0·8	5	—	2	—	—
P	22	2·6	110	122·4	22	1·6	0·8	5	—	5	—	—
Q	15	2·6	110	122·4	15	1·6	0·8	5	—	5	—	—
R	10	2·6	110	125·8	10	1·6	0·8	5	5	5	—	—
S	5	2·6	110	129·1	5	1·6	0·8	5	10	5	—	—
T	0	2·6	110	129·1	0	1·6	0·8	5	10	5	—	—

TABLE 2. Collected data of the effects of low sodium solutions (B–I Table 1) on the membrane potential and photoresponses of *Bufo bufo* and *Bufo marinus* rods. V_{pk} amplitude of peak of responses to saturating flashes, V_{pl} level of the subsequent plateau, V_m change in the resting membrane potential induced by ionic substitution. N is the number of units successfully recorded. In a large number of the reported cases the effects showed good reversibility

	V_{pk} (mV)	V_{pk}/V_{pl}	V_{pl} (mV)	ΔV_m (mV)	V_{pk} (mV)	V_{pk}/V_{pl}	V_{pl} (mV)	ΔV_m (mV)	V_{pk} (mV)	V_{pk}/V_{pl}	V_{pl} (mV)	N
A → B → A												
Mean	22·4	1·8	13	−6·2	15·7	2·2	7·9	+7·3	21·4	1·8	13·2	13
S.D.	3·6	0·3	4·1	1·55	4·6	0·5	4·2	2·4	6·1	0·4	6·4	
A → C → A												
Mean	26	2	13·4	−12·1	9·8	3·2	3·5	+14·3	25·3	2·0	13·5	19
S.D.	5	0·3	4·6	3·7	3·7	1·2	1·9	5	4·1	0·3	4·6	
A → D → A												
Mean	23·1	2·7	5·5	−9·2	1·7	—	0·5	+12·2	14·5	2·8	5·2	4
S.D.	2·8	0·1	0·8	3·3	1·5	—	0·5	3·6	3·1	0·5	0·8	
A → E → A												
Mean	22·9	2·1	11·4	−16	0·9	—	—	+18·1	27·4	2·2	10·1	14
S.D.	4·3	0·3	2·9	4·5	0·4	—	—	4·5	5·6	5·6	3·6	
A → F → A												
Mean	27·2	2·0	13·6	−22·3	0·5	—	—	+21·2	21·3	2	10·5	8
S.D.	4·2	0·3	3·3	3·6	0·3	—	—	3	4·7	0·2	1·8	
A → G → A												
Mean	25·3	2·1	12·1	−21·1	—	—	—	+20·3	24·1	2·2	11	5
S.D.	4·7	—	3·7	2·7	—	—	—	2·2	2·8	—	1·6	
A → H → A												
Mean	26·7	2·1	13·2	−28·2	—	—	—	+34·3	26·9	2	14·2	9
S.D.	3·9	—	5·2	3·7	—	—	—	5·6	3·8	—	5·2	
A → I → A												
Mean	23·6	2·1	11·3	−25·4	—	—	—	+29·4	20·4	2·1	9·8	14
S.D.	2·9	—	1·9	6·7	—	—	—	7·3	4·2	—	2·1	

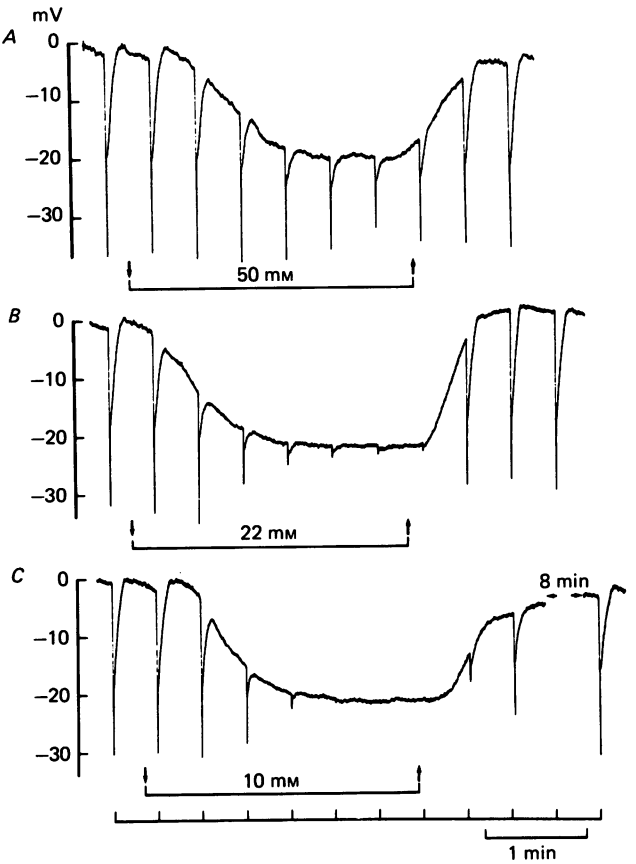

Fig. 1. Effects of low sodium concentrations on resting potential and photoresponses of a rod of *Bufo bufo*. Bars at the bottom of each record indicate the duration of exposure to low sodium solutions: 50 mM in *A*, 22 mM in *B* and 10 mM in *C*. The trace at the bottom of the Figure monitors the occurrence of a 20 msec flash of monochromatic light (510 nm) of photon density $1{\cdot}1 \times 10^{10}$ photons cm^{-2}.

transmembrane potential in the dark increased and reached a value close to that of the plateau of the control photoresponse. These results are consistent with previous findings indicating that in the dark the membrane potential of both cones (Cervetto, 1973) and rods (Brown & Pinto, 1974) is controlled by the external sodium concentration. It is also apparent that upon substitution of sodium by choline the peak of the maximal light response became less negative and the subsequent plateau hyperpolarized only slightly. These effects led to photoresponses of smaller size in which the ratio between peak and plateau was increased. Concentrations lower than 50 mM further hyperpolarized the rod membrane and eventually led to a complete blockage of the photoresponse (Fig. 1*B* and *C*). The value of the external Na^+ concentration at which photoresponses were undetectable was between 20 and 10 mM for thirty-one rods.

The record of Fig. 1*C* also illustrates the time course of recovery of both membrane potential and photoresponses from very low sodium concentration. The amplitude

of the photoresponse was still considerably reduced after a relatively fast depolarization that partially restored the original resting potential. Subsequently the membrane potential slowly returned toward the control level and the peak amplitude approached the original value.

Experiments described above show that light responses can be blocked by lowering the external sodium to about 20 mM. We may suppose that under these circumstances the light modulated current ΔI is suppressed. If we assume that $\Delta I = \Delta I_{Na} = \Delta g_{Na} \cdot (E_{Na} - V_m)$ conditions for $\Delta I = 0$ are realized either if $E_{Na} = V_m$ or if upon low

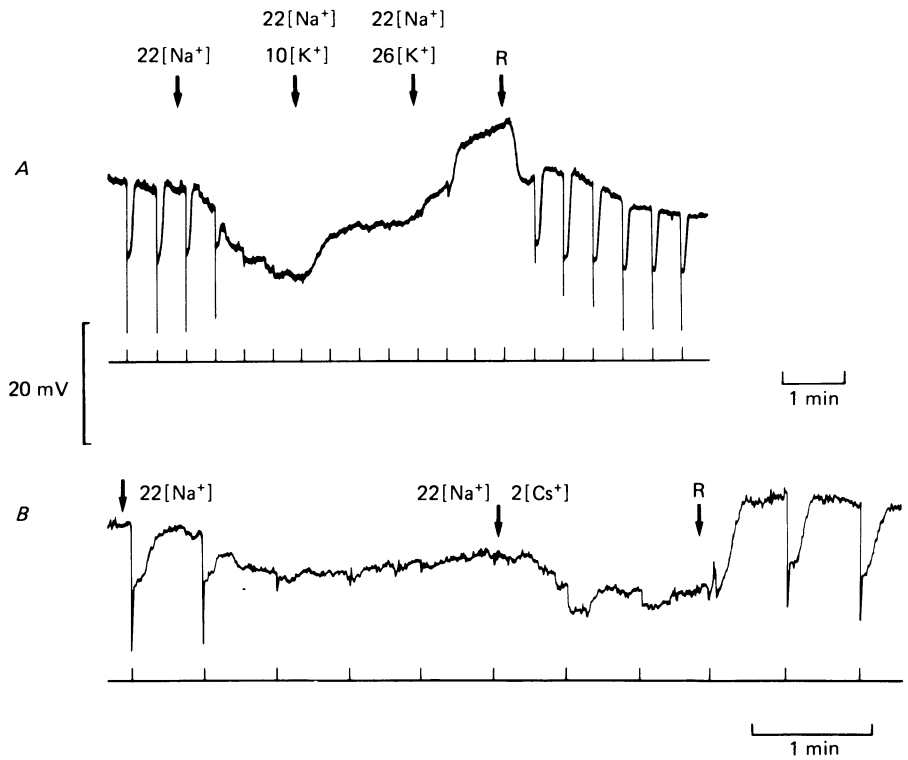

Fig. 2. Effects of low Na^+ (22 mM) and high K^+ (10 and 26 mM) on membrane potential and photoresponse of a rod of *Bufo marinus* (*A*). Comparison between the effects of 22 mM-Na^+ in the absence and in the presence of 2 mM-Cs^+ on membrane potential and photoresponses of a different rod of *Bufo marinus* (*B*). Arrows indicate the time of solution changes. The traces at the bottom of each record monitor light flashes whose intensity and duration was the same as in Fig. 1. R = washing in Ringer control solution.

external sodium $\Delta g_{Na} = 0$. In the first case further reduction of the external sodium should lead to photoresponses with inverted polarity. This prediction however is not satisfied even for nearly complete sodium substitutions. The assumption given above may also be tested by displacing V_m while keeping E_{Na} constant. Voltage responses to light should be restored upon membrane hyperpolarization below E_{Na} and responses of opposite polarity should appear as depolarization above E_{Na}.

The record in Fig. 2*A* was taken from a rod exposed to a sequence of different ionic media. At first the retina was bathed with a solution containing 22 mM-Na^+ (soln.

E), then 10 and 26 mM-K^+ were applied while keeping the Na^+ concentration at 22 mM (solutions J and K respectively). Under the influence of low sodium the membrane hyperpolarized and light responses vanished. Under those circumstances, membrane depolarizations induced by changes of E_K (ΔE_K of 33 and 58 mV respectively) failed to re-establish any detectable voltage response to flashes. The record in Fig. 2*B* was taken from a different rod and shows that application of 2 mM-Cs^+ in the presence of 22 mM-Na^+ induced hyperpolarization accompanied by a partial recovery of voltage photoresponses. This effect indicates that the blockage of photoresponses

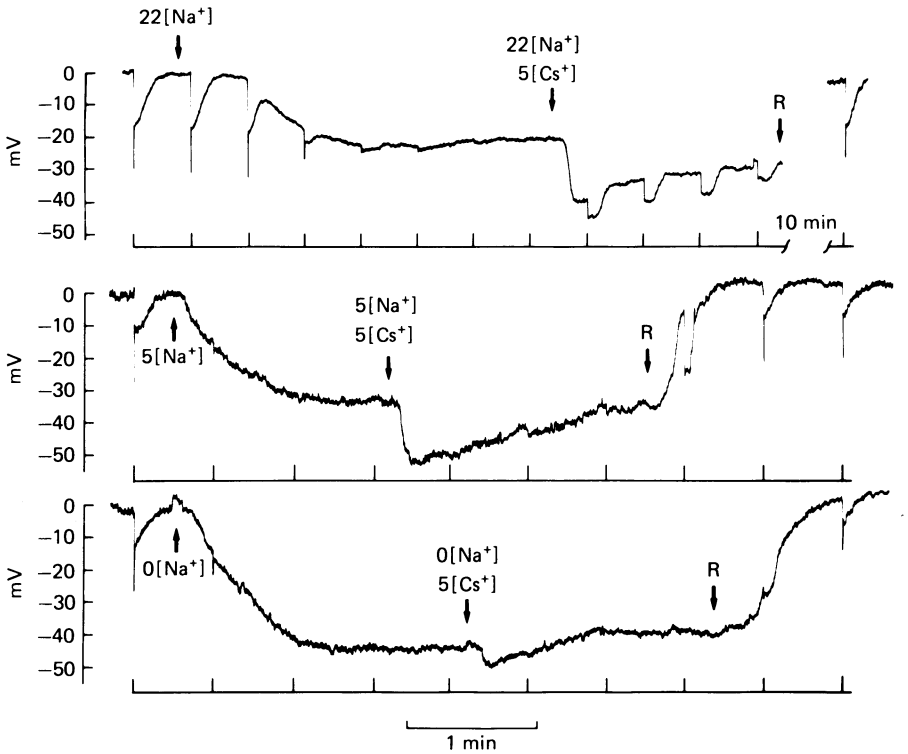

Fig. 3. Effects of 5 mM-Cs^+ on membrane potential and photoresponses of rods exposed to 22, 5 and 0 mM external sodium. The three records were obtained from retinae of *Bufo bufo*; the middle and bottom records belong to the same rod. Light stimuli as in the preceding Figures.

observed in low sodium cannot be merely attributed to a failure of the light-sensitive conductance mechanism. If we assume that the membrane hyperpolarization induced by low external sodium activates a parallel conductance sufficiently large to reduce the voltage effects of the light-dependent current to undetectable levels, the results obtained with caesium can be simply explained as the consequence of a reduced shunt.

Fig. 3 illustrates on two different rods the effects of 22, 5, 0 mM-Na^+ in both the absence and presence of 5 mM-Cs^+. The exposure to 22 mM-Na^+ ($\Delta E_{Na} = -45$ mV) hyperpolarized the membrane by about 20 mV and blocked photoresponses. Subsequently, the application of 5 mM Cs^+ further hyperpolarized the membrane by 16 mV and induced reappearance of light responses with modified kinetics.

TABLE 3. Influence of 5 mM-Cs^+ on membrane potential and photoresponses of rods exposed to low sodium test solutions

		22 mM-Na^+					
		Solution E		Solution O		Control	
	Control						
Cell	V_{pk} (mV)	V_m (mV)	V_{pk} (mV)	V_m (mV)	V_{pk} (mV)	V_m (mV)	V_{pk} (mV)
N22A2CS	29	−20·3	0	−18	6	—	24·2
		15 mM-Na^+					
		Solution F		Solution Q		Control	
	Control						
N15D02CS	25	−21·8	1	−16	2	—	—
N15D03CS	25	−18·5	1·2	−18	2	—	—
N15D04CS	28	−23·6	0·6	−24	—	+20	10
N15D05CS	26	−27	0	−23	2	+40	25
N15D06CS	22	−20	0	−13	1	+32	16
N15D10CS	26	−28	1	−18	1	+34	23
Mean	25·3	−23·2	0·6	−18·7	1·6	+31·5	18·5
S.D.	2	3·8	0·5	4·2	0·6	8·4	6·9
		5 mM-Na^+					
		Solution H		Solution S		Control	
	Control						
N05C1CS	26	−33·5	0	−16	1	+50	25·1
5CS5N1	28	−40	0	−17	0	+50	30
N05C2CS	23	−29·5	0	−15	6	+30	22
N05C3CS	28	−24·3	0	−15	5	+32	28
5CS5N2	19	−20	0	−10	2	+25	19
N05C6CS	25	−26	0	−10	3	+42	25
N05C7CS	21	−23	0	−10	1	+23	16
N05C8CS	28	−32	0	−18	4	+34	27
Mean	24·8	−28·5	0	−13·9	2·8	+35·8	24
S.D.	3·5	6·5	0	3·4	2·1	10·5	4·7
		0 mM-Na^+					
		Solution I		Solution T		Control	
	Control						
5CS0N1	20	−29	0	−6	0	+40	17
N0B3CS	20	−26	0	−5	3	+35	20
5CS0N2	20	−34	0	−8	2	+35	19
N0B2CS	29	−29	0	−8	3	+40	25
N0B8CS	24	−28	0	−4	3	+32	19
5CS0N3	20	−28	0	−4	1	+31	16
N0B10CS	28	−23	0	−8	4	+30	23
N0B5CS	24	−38	0	−5	0	+40	20
Mean	23·1	−29·4	0	−6	2	+35·4	19·9
S.D.	3·8	4·7	0	1·8	1·5	4·2	2·9

No response whatsoever was detected when the external sodium was brought to 5 mM or less. In 5 mM-Na^+ ($\Delta E_{Na} = -82$ mV) the membrane hyperpolarized by about 35 mV, thus approaching the maximal peak internal negativity of responses to saturating flashes. Application of caesium in these conditions further hyperpolarized by about 14 mV. In these conditions small responses to light stimuli could be detected upon averaging. Collected data are reported in Table 3. Taking into account that in the example described in Fig. 3 the resting potential recorded upon withdrawal of

the micro-electrode was 36 mV, the maximal hyperpolarization that the membrane potential reached in low sodium and caesium was −85 mV. This level however was not maintained and the membrane depolarized at a rate of 5·5 mV per min. The phenomenon consistently appeared in all experiments (eight rods); in a single case, low sodium conditions in the presence of caesium were maintained long enough to observe that the membrane returned to the levels of potential it had before application of caesium. Under those conditions however the internal ionic concentration of the rod was probably modified. If by the effects of both low sodium and presence of caesium the shunting currents across the membrane are drastically reduced one may expect even a small current to become apparent. This may also include a current associated with a metabolic pump.

When the external sodium was completely replaced by choline, application of caesium still hyperpolarized the membrane by some 10–15 mV, an effect that cannot be attributed to inactivation of sodium channels. Even under similar conditions on some occasions small light responses reappeared as observed by averaging several sweeps. It is not known if this is the result of presence of residual sodium or is an indication of more complex phenomena.

Selectivity of the light-dependent channels

The results of sodium substitution of the experiments described so far can be explained either assuming that only sodium ions can permeate through light-sensitive channels or assuming that two or more distinct ionic species can move through. The first assumption implies that the equilibrium potential for the photoresponse coincides with E_{Na}. One may conceive that under normal conditions or in low external sodium the effects of a light-modulated conductance to an ionic species distinct from sodium are concealed by the presence of shunting conductances and/or because the driving forces are small. A way to emphasize an hypothetical contribution to the photocurrent other than the sodium component is to make the latter negligible. The experiments shown in Fig. 4 represent such an attempt. The specific aim was to check whether a current carried by potassium could be modulated by light: the retina was exposed to solutions in which sodium was replaced in various proportions by equimolar amounts of potassium. TEA, cobalt and caesium were sometimes added to the test solution in order to minimize the effects of voltage-dependent conductances. The record illustrated in Fig. 4*A* was obtained from a rod during the exposure to 40 mM-Na^+ and 90 mM-K^+, a condition that corresponds to a $\Delta E_{Na} = -30$ mV and a $\Delta E_K = 89$ mV. It is seen that the membrane depolarized by about 40 mV (average value 35 ± 5 mV from six experiments) thus approaching a steady value near zero.

Under those circumstances no voltage change associated with light could be detected. Fig. 4*B* illustrates the effects of a solution in which sodium was completely replaced by potassium with a $\Delta E_K = 96$ mV and a ΔE_{Na} indeterminate. Under these circumstances the membrane depolarized by about 40 mV suggesting that g_{Na}/g_K was small. In these conditions it is likely that the ionic gradients were inverted, that is $E_K > V_M > E_{Na}$. Therefore one would expect either hyperpolarizing or depolarizing photoresponses depending on whether the action of light was to decrease or increase g_K respectively. In contrast to these predictions no light responses were detected as checked also by averaging. The effects of a sodium-free and high potassium solution

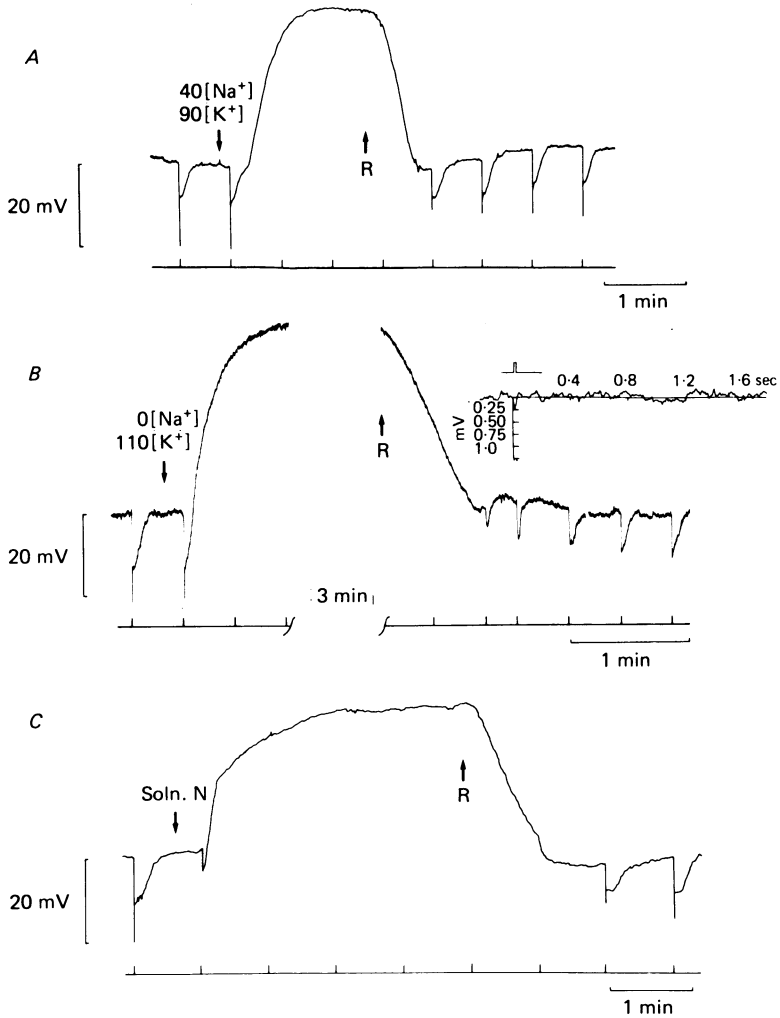

Fig. 4. Effects of different $[Na^+]_o/[K^+]_o$ upon membrane potential and photoresponses of three different rods (*Bufo bufo* in *B*, *Bufo marinus* in *A* and *C*). In *A* $[Na^+]_o = 40$ mM, $[K^+]_o = 90$ mM. In *B* $[Na^+]_o = 0$, $[K^+]_o = 110$ mM. In *C* the high-potassium, low-sodium solution also contained TEA, Cs^+ and Co^{2+}. The interruption in record *B* corresponds to a 3 min period during which nine sweeps were averaged. Flashes are monitored at the bottom of each record and their intensity was as in Fig. 1. The inset in *B* shows the average of ten responses. No significant voltage change is detectable.

(solution N, Table 1), containing 10 mM-TEA, 1 mM-$CoCl_2$ and 5 mM-CsCl are illustrated in Fig. 4*C*. In high external potassium E_K is around 0 mV and the membrane dark potential is in all circumstances close to 0 mV, suggesting the existence of a very large potassium conductance. This potassium current could shunt powerfully the photocurrent leading to a negligible photovoltage response. In the inset of Fig. 4*B* the average response to a very bright flash of light (ten responses) in solution M is presented and no detectable photoresponse is present. Therefore it is seen that high external potassium in the absence of sodium is associated with no

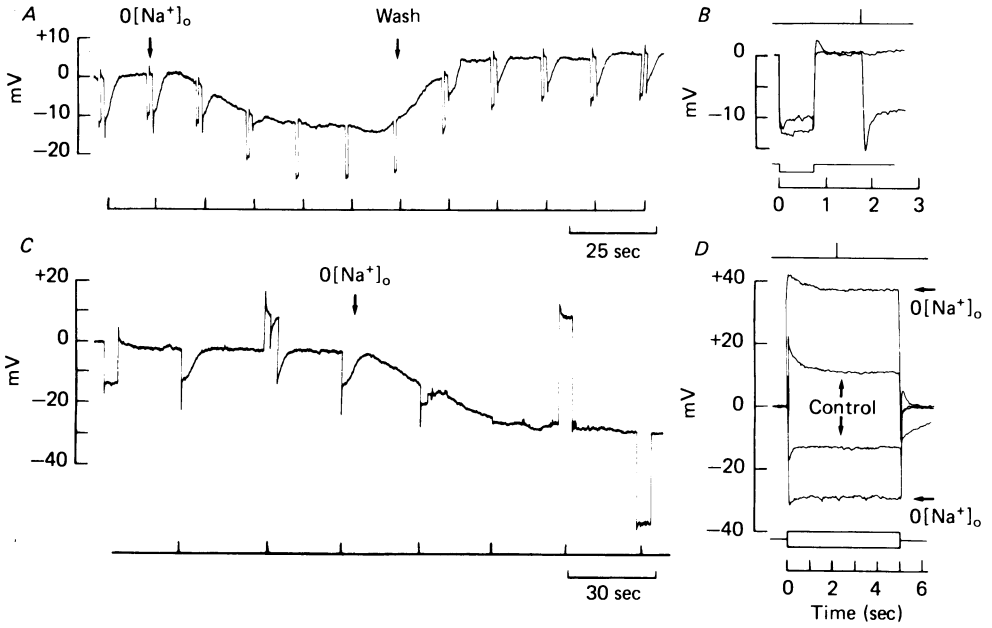

Fig. 5. Input resistance changes in two different rods of *Bufo bufo* exposed to Na^+-free test solution. Light monitors at the bottom of records on *A* and *C* and above records in *B* and *D*. Raised bars under records in *B* and *D* are current monitors. *A*, pulses of inwardly directed current ($2{\cdot}2 \times 10^{-10}$ A, 720 msec) were injected through the recording electrode 1700 msec before illumination, during exposure of the retina to nominally 0 Na^+. *B*, superposed records of responses to current and light in control and 0 Na^+ solutions. *C*, responses at enlarged scale are the same as in the record in *A*. 5 sec, $2{\cdot}2 \times 10^{-10}$ A pulses of both inward and outward current applied in both darkness and during light responses, during exposure to nominally 0 Na^+. *D*, superposed records of current responses in normal and low sodium obtained from the same cell as in *C*.

voltage photoresponse even under conditions that supposedly reduce the effects of shunting currents (Fain *et al.* 1978; Fain & Quandt, 1980).

Conductance changes in low external sodium

Fig. 5 summarizes the changes of the input conductance recorded in a rod exposed to low sodium. In Fig. 5*A* the response of a rod to a short pulse of inwardly directed (negative) current occurs in the dark intervals between photoresponses. The decrease in size of photoresponses upon exposure to low sodium is associated with a decrease of the input conductance of the cell. In Fig. 5*B* superposed records illustrate on an expanded time scale responses to current and light in both normal conditions and low external sodium. Pulses of both positive and negative current were applied to a rod whose intracellular activity is illustrated in Fig. 5*C* in the absence of sodium. A flash response superposed to the membrane depolarization induced by a positive pulse of current in normal external sodium appears reduced in size compared to control responses. Both positive and negative currents induced significantly larger potential drops in low sodium than in control conditions. Photoresponses were not restored by positive currents in low external sodium. Voltage responses to positive

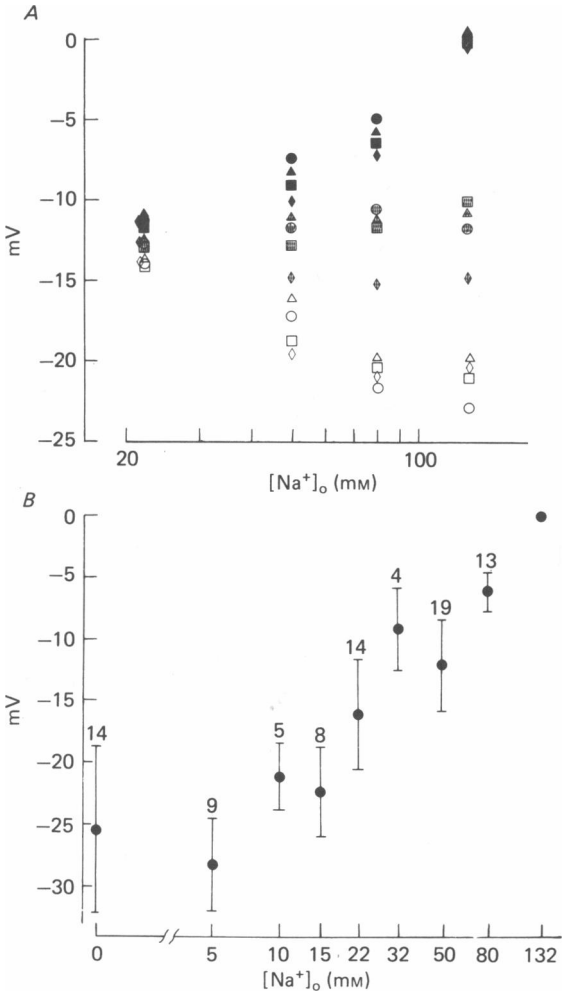

Fig. 6. *A*, relations between membrane potential in the dark (filled symbols), at the plateau (shaded symbols), and at the peak (open symbols) of responses to bright flashes (see Fig. 1) and log $[Na^+]_o$ in four rods of *Bufo marinus*. *B*, relation between dark potential and log $[Na^+]_o$ in rods of *Bufo bufo*. Data points are averaged values of dark voltage displacements from control conditions computed for the indicated numbers of cells. Bars are ±S.D.

and negative pulses from the same experiment as in Fig. 5*C* are shown on an expanded time scale in Fig. 5*D*. It is seen that the shape of responses to pulses of current is changed by low sodium. The prominent sag present in responses evoked in control conditions by positive and negative pulses was drastically reduced in low sodium. This fact could suggest that the mechanism of rectification is dependent upon the sodium current. On the other hand the low external sodium hyperpolarized the membrane potential by 10–20 mV, possibly displacing the membrane potential in a range where the voltage-dependent conductance is already fully activated. Measurements of the input conductance performed by passing pulses of current through the recording electrode can be questioned for several reasons and especially because the

electrical coupling between rods and the rectifying properties of micro-electrodes prevent reliable measurement of membrane polarization. A clear-cut effect like that shown in Fig. 5 suggests that the input conductance drops to an extent that can be explained supposing that membrane conductance, and perhaps coupling between rods, decrease in low external sodium.

Relative values of the sodium conductance in the dark and during illumination could be estimated in principle from the relations between membrane potential and log of external sodium concentration. Similar relations are reported in Fig. 6. The membrane potential in the dark at the peak and plateau of responses to bright flashes are plotted in Fig. 6*A* for four different rods with very similar membrane potential and photoresponses. Dots in Fig. 6*B* represent the average value of the membrane potential in the dark from a large population of rods. The slope of the function is the steepest at the highest levels of the external sodium with a value of about 27 mV per log unit. If we assume that in this range of high external sodium g_K and g_{Na} are in first approximation constant and the voltage-dependent conductances are negligible we may estimate that the sodium conductance of the rod membrane in darkness is about half of the total membrane conductance. At intermediate and low levels of external sodium the curve becomes less steep and this may be due to rectification of the sodium channels and activation of voltage-dependent conductances or imperfect selectivity of sodium channels.

The plateau values of responses to bright flashes were only weakly influenced by changing the external sodium from the control concentrations to levels at which responses vanished. The corresponding peak levels, instead, moved to less negative values (see also Fig. 1).

The example reported below deviates somehow from the typical behaviour described so far and, although representative of a minority of cases, perhaps deserve mention. Responses to flashes of increasing intensity as recorded at three different external sodium concentrations are illustrated in Fig. 7. The responses to dim flashes had a relatively slow time course (the time to the peak exceeded 800 msec) and a relatively small initial transient. The average value of the ratio between peak and plateau for responses to saturating lights was usually close to 2 while in this example the ratio is 1·15. These features reflect perhaps the fact that potential-and time-dependent conductances contribute negligibly in this case to voltage responses (see Capovilla *et al.* 1980). The data plotted in Fig. 7*B* illustrate how lights of increasing intensity influence the relations between membrane potential and external sodium for the same cell. For dim lights the slope of the curves decreased proportionally to the estimated number of absorbed photons. A flash equivalent to an average of 3·3 absorbed photons decreased the slope from about 30·7 to 27·8 mV per log unit, and a flash four times brighter further reduced the slope to 16·2 mV.

If we assume that light primarily reduces sodium conductance we may estimate from these data the efficiency of a single photon in reducing the sodium conductance. We assume that voltage-dependent conductances contribute negligibly in this particular example to the slopes of the relations between membrane potential and external sodium, and that the slopes correspond to the relative sodium conductance (g_{Na}/g_{tot}).

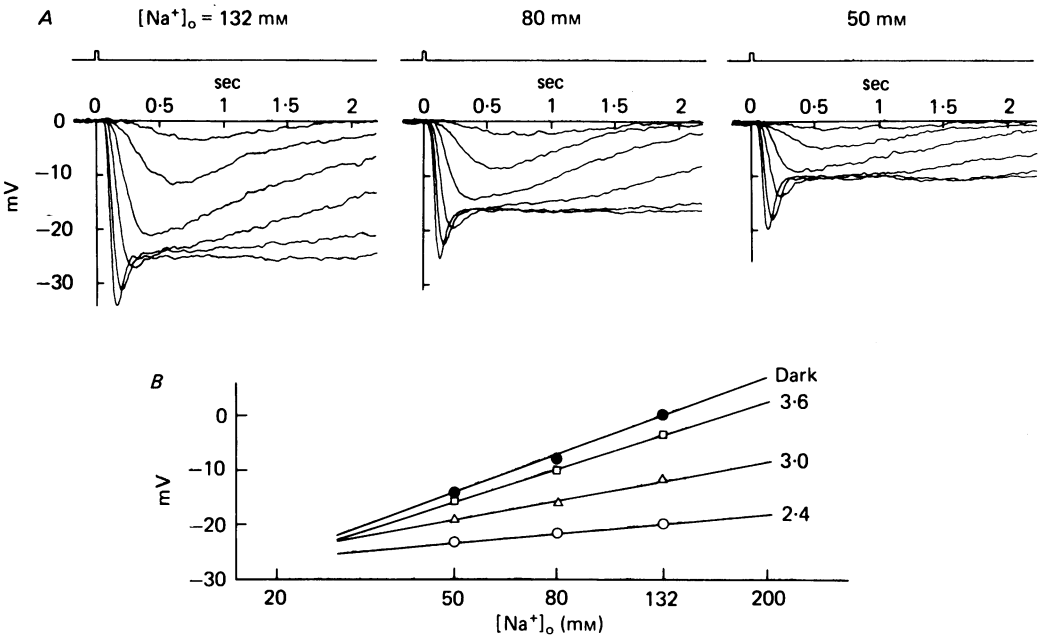

Fig. 7. *A*, photoresponses to flashes of increasing intensity in rods in normal medium and in test solution with 80 and 50 mM-Na^+. Light intensity was increased by steps of 0·6 log units. The dimmest flash delivered $5{\cdot}5 \times 10^8$ photons cm^{-2} sec^{-1} equivalent to 3·3 photoisomerizations per flash. *B*, relations between membrane potential and external sodium concentration at different light intensities for the same cell as in *A*. Light attenuations are indicated in log units by numbers at the right of each curve.

We define the fractional conductance change per isomerization as

$$\eta_g = \lim_{I \to 1} \frac{\Delta g_{Na}}{g_{Na}} \frac{1}{I}, \tag{1}$$

where I is the number of absorbed photons and

$$\Delta g_{Na} = g_{Na} - g^*_{Na} \tag{2}$$

with g^*_{Na} the sodium conductance in light. With the experimentally measured slopes in the darkness (S_1) and in the light (S_2) as:

$$S_1 = g_{Na}/g_{tot} \quad \text{and} \quad S_2 = g^*_{Na}/g^*_{tot} \tag{3}$$

(g_{tot} and g^*_{tot} are the total membrane conductances in darkness and light, respectively) we obtain

$$\frac{\Delta g_{Na}}{g_{Na}} = \frac{1 - S_2/S_1}{1 - S_2} \tag{4}$$

with $S_1 = 0{\cdot}53$ and $S_2 = 0{\cdot}48$ we obtain $\eta_g = 0{\cdot}074$. For a membrane conductance estimated as $\simeq 0{\cdot}6 \times 10^{-9}$ S (W. G. Owen & V. Torre in preparation), g_{Na} will be $0{\cdot}34 \times 10^{-9}$ S and a single photon will decrease this conductance by about $2{\cdot}5 \times 10^{-11}$ S. A value close to this has been obtained also from measurements of the photocurrent recorded from the outer segments (Baylor *et al.* 1979).

Dependence of the plateau on the external sodium concentration

With the experiments described above we have suggested that the plateau subsequent to the peak of responses to bright flashes is made slightly more negative by external sodium concentrations as low as 22 mM. This observation may lead to the conclusion that the sag from peak to plateau is relatively independent of the sodium current and that during periods of constant illumination the sodium conductance remains negligible. This is equivalent to saying that the potential and time-dependent conductance does not necessarily involve sodium. Indeed a similar conclusion has been recently reached by Werblin (1979). The subject, however, seems

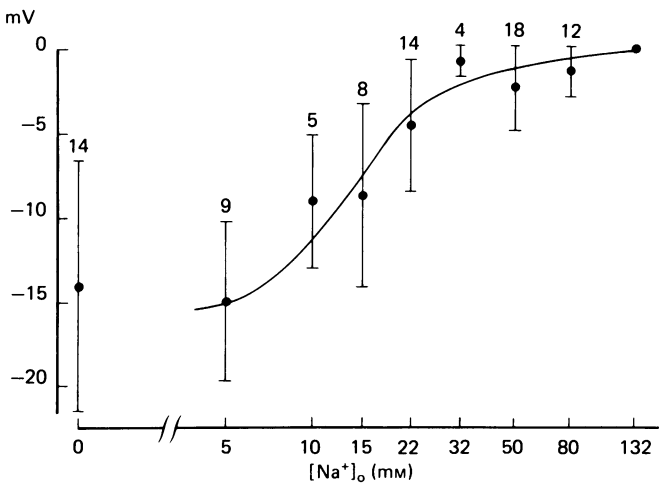

Fig. 8. Dependence of plateau potential upon $[Na^+]_o$. Light stimuli were 20 msec flashes delivering $5{\cdot}5 \times 10^{11}$ photons cm^{-2} sec^{-1}. The quantity (X of Fig. 9) plotted on the ordinates is the difference between plateau potentials in control conditions and at each sodium concentration. Data points are averaged values computed on the numbers of cells indicated. Bars are ±S.D. The line through data points was drawn by eye.

far more complex and we must distinguish between the effects observed at intermediate levels of sodium concentration and those observed at the lowest levels. In Fig. 8 the changes in plateau amplitude of responses to saturating flashes induced by changes in external sodium is plotted against the log of the external sodium concentration.

It is seen that the plateau negativity is slightly increased in the range between the control external sodium and 22 mM, and that much larger changes occur when the external sodium is further lowered. A different picture emerges when the influence of external sodium is explored upon the level of the plateau obtained with steady illuminations.

Fig. 9 illustrates the results of experiments performed to compare the effects of partial substitutions of the external sodium on the plateau of responses to bright flashes and to steps of light. The influence of 50 mM-Na^+ on responses to both flashes and steps is illustrated for the same cell in *A*. It is seen that the level of the plateau to flashes was only slightly increased upon sodium substitution (X, left column). Responses to sustained illumination were usually characterized by an initial transient

followed by a plateau slowly declining toward a less hyperpolarized value. Sodium substitutions performed 10–30 sec after the initiation of illumination induced appreciable increments of the membrane potential (Y, right column). The extent of those changes increased with the time of exposure to light. In *B* it is shown that the steady illumination initiated after nearly complete blockage of photoresponses by 22 mM-Na^+

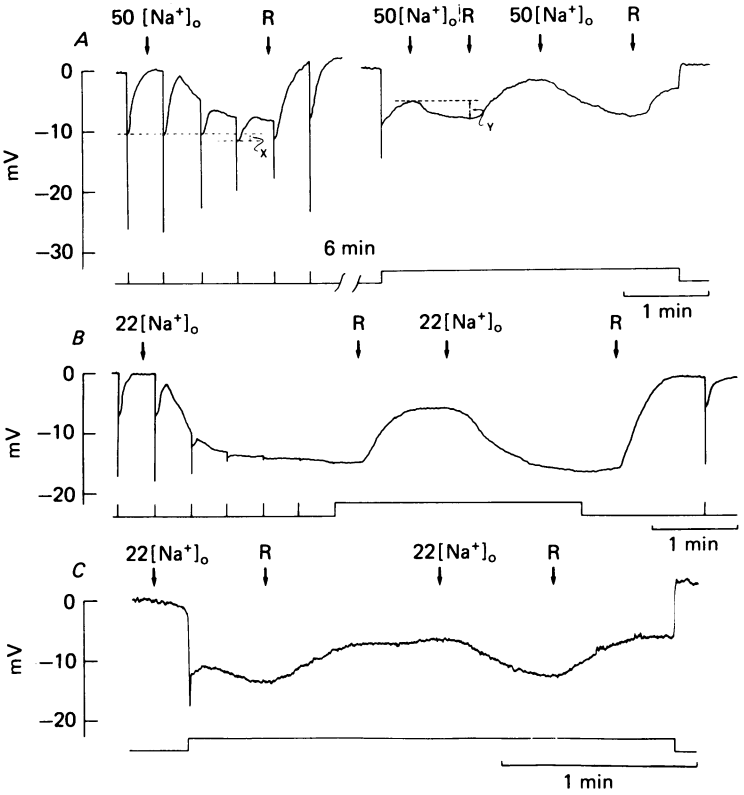

Fig. 9. Comparison between the effects of low external sodium on plateau of responses to flashes and steps of light. Light monitors shown at the bottom of each record. *A*, influence of 50 mM-Na^+ on plateau of responses to flashes (left) and to a step of light (right) on the same rod. Labels X and Y indicate how displacements of plateau potential were measured in the two conditions. *B*, effects of 22 mM-Na^+ on responses to flashes and on the membrane potential during steady illumination. *C*, effects of low Na^+ on membrane potential at different periods of time during steady illumination. The intensity of the flashes expressed as a number of photons cm^{-2} sec^{-1} was $5{\cdot}5 \times 10^{11}$. The intensity of the steps delivered in the experiment illustrated in *A* and *C* was $3{\cdot}5 \times 10^{10}$ photons cm^{-2} sec^{-1}. The intensity of the step presented in *B* was the same as that of flashes.

is associated with large potential changes upon changing the external sodium from normal values to 22 mM. The influence of 22 mM-Na^+ at different periods of steady illumination is illustrated in *C*. It is seen that the size of the membrane hyperpolarization induced at the late periods of steady illumination by low sodium on the plateau was larger than the hyperpolarization induced at the early periods. These differences may be interpreted as being due to an increased dependence of the membrane potential

on the external sodium that develops during periods of illumination. Relations between membrane potential and external sodium obtained after a prolonged exposure to light are illustrated in Fig. 10. The curve drawn through the experimental points is significantly different from that reported in Fig. 8 which is redrawn here (dashed line) for comparison. The data plotted in Fig. 10 have been obtained with steps of light delivering from 3·5 to 55×10^{10} photons cm^{-2} sec^{-1} and with changes

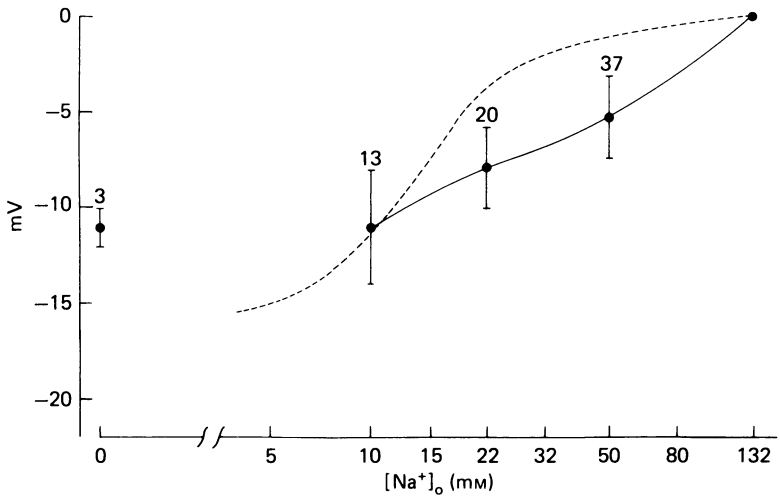

Fig. 10. Changes in membrane potential induced by low sodium 20 or more seconds from the onset of a steady illumination. Data points are averaged values of the quantity Y (see Fig. 9*A*) measured in different cells. Bars are ±S.D. Number of samples are indicated for each data point. The continuous line through data points was drawn by eye. Dashed line is the same as in Fig. 8 redrawn for comparison.

of external sodium initiated 20–40 sec after the onset of the steady light. We have not studied in detail the dependence of the curve drawn in Fig. 10 on the light intensity and on the time of exposition to low external sodium. However, these observations suggest that upon a prolonged illumination the relative value of the light-sensitive conductance to sodium increases. The analysis of this effect is deferred to a following paper (M. Capovilla, L. Cervetto, E. Pasino & V. Torre, in preparation).

DISCUSSION

The results in this paper indicate that the ionic current which is directly modulated by light depends primarily on the external sodium concentration and suggest that the current associated with the voltage- and time-dependent processes has multiple components.

Nature of the photocurrent

A quantitative account of the properties of the current controlled by light at the outer segments of rods has been recently given by Baylor *et al.* (1979). These authors indicate that a single photon may decrease the 'dark current' by about 1×10^{-12} A and that a saturating flash shuts off a current of about 3×10^{-11} A. The question is whether these changes can be accounted for by sodium current alone. The results

reported above suggest that light-dependent current is carried primarily by sodium and show no indication of the existence of a light-modulated potassium current. In addition our estimate of the single quantum efficiency of the sodium conductance gives the value of $2{\cdot}5 \times 10^{-11}$ S, which is in excellent agreement with the estimate of 3×10^{-11} S based on measurements of the photocurrent (Baylor *et al.* 1979). Thus the indications emerging from the present work seem to support the view that in a variety of conditions the current modulated by light is carried primarily by sodium. One cannot exclude, however, that under special circumstances light may activate a small current to ions other than sodium. This could explain the small but consistent recovery of voltage responses in the presence of caesium when the external sodium was nominally zero.

At the lowest values of the external sodium and in the presence of caesium the conditions should be realized for $E_{Na} < V_m < E_K$. Under these circumstances in the absence of large shunting conductances a decrease of g_{Na} should produce a depolarizing response. The failure of the occurrence of an inverted response in low external sodium and high potassium cannot be attributed to a permanent block of the light-gated mechanism due to low sodium, since light responses can be obtained in very low external sodium in the presence of caesium (see Fig. 3). A simple explanation for not observing photoresponses with inverted polarity could be that the light-sensitive channels have rectifying properties in low external sodium, or that in those conditions the efflux of Na^+ is masked by the flow of other ions through the light sensitive channels. Also it is necessary to consider the possibility of a relatively slow ionic change, which would let the ionic concentration re-equilibrate or the possibility that high external potassium blocks the light-sensitive channel.

The assumption that E_{Na} coincides with the equilibrium potential for the photoresponse implies that an unusually high level of Na^+ is contained within the rod. This may raise questions on how the cell can maintain constant volume and electrical neutrality. In a previous study we have shown that at the peak of light responses rods in high external potassium approach the behaviour of a potassium electrode (Capovilla *et al.* 1980). Considering that in 26 mM-K^+ rods depolarize on average 16 mV and still produce a 6 mV hyperpolarizing response to light we find that:

$$E_K = V_D + 16 \text{ mV} - 6 \text{ mV} - 58 \text{ mV}$$

and with $V_D = -38 \pm 10$ mV, $E_K = -86 \pm 10$ mV.

Accordingly 117 mM $\geqslant [K^+]_i \geqslant$ 53 mM. Considering then that in the presence of caesium photoresponses are nearly blocked when $[Na^+]_o = 5$ mM, that $\Delta E_{Na} = 82$ mV and the corresponding $\Delta V_m = -40$ mV, we obtain

$$E_{Na} = \Delta E_{Na} + V_D + \Delta V_m = 4 \pm 10 \text{ mV},$$

140 mM $\geqslant [Na^+]_i \geqslant$ 76 mM.

The estimation of E_{Na} and $[Na^+]_i$ is based on the assumption that the light-sensitive channel is perfectly selective to Na^+ ions. If the light-sensitive channel is not perfectly Na^+-selective then E_{Na} can be higher and $[Na^+]_i$ lower.

Properties of the voltage-dependent processes

A description of the voltage- and time-dependent properties of the rod membrane has been given recently by Bader *et al.* (1980) in a voltage-clamp study performed on solitary rods. As already suggested by Fain *et al.* (1978) the depolarizing sag in response to bright flashes is associated with a conductance increase. Additional information on the ionic nature of this effect comes from the results described above that show a relative independence of the level of the plateau on the external sodium. The data illustrated in Fig. 8 may suggest that the ionic channels responsible for the current associated with the depolarizing sag is selective to cations. In other words the membrane might be shunted by unspecific channels. Alternatively, one may suppose that the voltage-dependent channel is specific for sodium ions, but that the current flowing through saturates above a relatively low concentration of external sodium. Accordingly a reduction of the driving forces corresponding to a ΔE_{Na} of 47 mV when the sodium is reduced to 20 mM may not significantly interfere with the current. Further reductions of the external sodium however may be effective in decreasing the current. In physical terms this behaviour may be explained assuming that the conductance of the voltage-sensitive channel depends on the presence of external sodium that may bind to specific gating sites and limit the current. If we suppose that these sites have more affinity for caesium than for sodium ions we could easily explain the effects of very low external sodium and the effects of caesium as well. A channel of this type may also be described as a multi-ion single file pore (see Hille & Schwarz, 1978).

The observation that application of caesium still hyperpolarizes the rod membrane when the external sodium is nominally zero suggests that caesium does not block only sodium channels. It may be that in those conditions E_{Na} is more negative than E_K and that caesium affects also g_K. The transient nature of this effect could be due to a decreased pumping rate following a decrease of the internal concentration of sodium ions. An alternative possibility could be that caesium induces a transient activation of the pumping rate whose effect on the membrane potential becomes apparent when the membrane resistance is sufficiently high.

Comparing the effects of low external sodium on the amplitude of the plateau of responses to flashes and steps it is seen that the sodium conductance in the two conditions is different. In a following paper we propose that with a steady light, sufficiently bright to close the light-sensitive channels nearly completely, a process develops which leads to the opening of a significant fraction of light-sensitive sodium channels.

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REFERENCES

BADER, C. R., MACLEISH, P. R. & SCHWARTZ, E. A. (1979). A voltage clamp study of the light response in solitary rods of the tiger salamander. *J. Physiol.* **296**, 1–26.

BAYLOR, D. A., LAMB, T. D. & YAU, K.-W. (1979). Membrane current of single rod outer segment. *J. Physiol.* **288**, 589–611.

BROWN, J. E. & PINTO, L. H. (1974). Ionic mechanism for the photoreceptor potential of the retina of *Bufo marinus*. *J. Physiol.* **236**, 575–591.

CAPOVILLA, M., CERVETTO, L. & TORRE, V. (1979). Quantum sensitivity of sodium conductance in toad rods. *J. Physiol.* **295**, 27*P*.

CAPOVILLA, M., CERVETTO, L. & TORRE, V. (1980). Effects of changing the external potassium and chloride concentrations on the photoresponses of *Bufo bufo* rods. *J. Physiol.* **307**, 529–551.

CAVAGGIONI, A., SORBI, R. T. & TURINI, S. (1973). Efflux of potassium from isolated rod outer segments: a photic effect. *J. Physiol.* **232**, 609–620.

CERVETTO, L. (1973). Influence of sodium, potassium and chloride ions on the intracellular responses of turtle photoreceptors. *Nature, Lond.* **241**, 401–403.

CERVETTO, L., PASINO, E. & TORRE, V. (1977). Electrical responses of rods in the retina of *Bufo marinus*. *J. Physiol.* **267**, 17–51.

DETWILER, P. B., HODGKIN, A. L. & MCNAUGHTON, P. A. (1980). Temporal and spatial characteristics of the voltage response of rods in the retina of the snapping turtle. *J. Physiol.* **300**, 213–250.

FAIN, G. L. & QUANDT, F. N. (1980). The effects of tetraethylammonium and cobalt ions on responses to extrinsic current in toad rods. *J. Physiol.* **303**, 515–534.

FAIN, G. L., QUANDT, F. N., BASTIAN, B. L. & GERSCHENFELD, H. M. (1978). Contribution of a caesium sensitive conductance increase in the rod photoresponse. *Nature, Lond.* **272**, 467–469.

HAGINS, W. A., PENN, R. D. & YOSHIKAMI, S. (1970). Dark current and photocurrent in retinal rods. *Biophys. J.* **10**, 380–412.

HILLE, B. & SCHWARZ, W. (1978). Potassium channels as multi-ion single-file pores. *J. gen. Physiol.* **72**, 409–442.

KORENBROT, J. I. & CONE, R. A. (1972). Dark ionic flux and the effects of light in isolated rod outer segments. *J. gen. Physiol.* **60**, 20–45.

SILLMAN, A. J., ITO, H. & TOMITA, T. (1969). Studies of the mass receptor potential of the isolated frog retina. I: General properties of the response. *Vision Res.* **9**, 1435–1442.

WERBLIN, F. S. (1979). Time- and voltage-dependent ionic components of the rod response. *J. Physiol.* **294**, 613–626.