Ionic Dependence of Reversal Voltage of the Light Response in *Limulus* Ventral Photoreceptors

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ABSTRACT The light-induced current as measured using a voltage clamp (holding voltage at resting potential) is attenuated when sodium ions in the bathing solution, Na_o, are replaced by Tris, choline, or Li or when NaCl is replaced by sucrose. After replacement of NaCl by sucrose, the reversal voltage, V_{rev}, for the light response becomes more negative. In this case, the slope of the V_{rev} vs. log Na_o near Na_o = 425 mM is approximately 55 mV/decade increase of Na_o (mean for 13 cells). The slope decreases at lower values of Na_o. Choline is not impermeant and partially substitutes for Na; the slope of V_{rev} vs. log Na_o is 20 mV/decade (mean for three cells). V_{rev} does not change when Na is replaced by Li. Decreases in the bath concentrations of Ca, Mg, Cl, or K do not affect V_{rev}. When Na_o = 212 mM, V_{rev} becomes more positive when K_o is increased. Thus, light induces a change in membrane permeability to Na and probably also to K.

INTRODUCTION

Photoreceptor cells of the ventral rudimentary eye of *Limulus* respond to light by generating positive-going receptor potentials. By a voltage clamp technique, the net membrane current associated with the light response has been measured; this light-induced current is inward (positive) and reverses sign when membrane voltage is displaced more positive than +10 to +20 mV, when measured in artificial seawater (ASW) (Millecchia and Mauro, 1969 *b*; Lisman and Brown, 1971). Millecchia and Mauro (1969 *a*, *b*) attempted to determine which ions carried this inward light-induced current by removing first one and then another of the ions from the ASW. They reported that with total replacement of extracellular sodium ions by Tris, choline, or lithium ions, the light response was attenuated but not abolished, that with removal of calcium ions the light response became larger, and that magnesium, potassium, and chloride ions had no direct effect on the am-

plitude of the light response. They also reported that the reversal voltage of the light-induced current was 10–15 mV more negative in sodium-free (Tris) seawater than in ASW; exchanges of ions other than sodium did not lead to changes in reversal voltage (Millecchia and Mauro, 1969 *b*). They proposed that sodium ions were the principal species carrying the light-induced current, but that other ions might participate.

If light induces an increase in membrane permeability to sodium ions exclusively, the reversal voltage (V_{rev}) should be equal to the Nernst equilibrium potential for Na. If light induces changes in membrane permeabilities to more than one monovalent cation then an equation of the form

$$V_{rev} = \frac{RT}{F} \cdot ln \frac{[\Sigma\ \Delta P_i \cdot [i]]_{out}}{[\Sigma\ \Delta P_i \cdot [i]_{in}},$$

where ΔP_i is the change in permeability in the i^{th} ion, might be a better approximation for reversal voltage (cf. Chandler and Meves, 1965; Hille, 1972). To reexamine this question, we have changed the extracellular ion concentration for sodium, potassium, calcium, magnesium, and chloride and determined V_{rev} as a function of ion concentration. A preliminary note on some of our results has previously appeared (Brown and Mote, 1971).

METHODS

The ventral rudimentary eye of *Limulus polyphemus* (Demoll, 1914; Clark et al., 1969) was dissected and mounted in a small channel in silicone rubber (Sylgard 184, Dow Corning Corp., Midland, Mich.); the channel had a volume of about 0.2 ml. The bathing seawater flowed over the eye at a rate of 1–2 ml/min. The voltage clamp, recording methods, and stimulator have been described previously (Lisman and Brown, 1971). The reference electrode was an Ag-AgCl wire inserted into a 3-mm glass tube filled with 3 M KCl + 2% agar, and with the lumen constricted to about 50 μm at the end. Compositions of the stock seawater solutions are given in Table I. The concentration of an ion in a test solution was adjusted by mixing a stock solution with ASW in varying proportions. For those cells (of Fig. 3) from which reversal voltage at Na_o = 637 mM was measured, all the test solutions at lower Na concentrations contained an additional amount of sucrose to make the solutions osmotically equivalent.

We determined reversal voltage of the light-induced current in the following way. Stimuli of constant intensity and duration were given every 30 s. The command voltage of the voltage clamp was moved slowly from resting voltage to a more positive value preceding a stimulus and slowly returned to resting voltage after the stimulus. At progressively more positive membrane voltages, the light-induced current became smaller and at some voltage was approximately zero. For more positive voltages the light-induced current reversed sign and became larger (Fig. 1 *a*). We plotted the light-induced current vs. membrane voltage (as in Fig. 1 *b*) and interpolated to determine the voltage, V_{rev}, at which the current was zero. During an experiment, V_{rev} was determined in ASW, then the bathing solution was rapidly

TABLE I

COMPOSITION OF SOLUTIONS

(Values in moles per liter)

Solution	NaCl	KCl	$MgCl_2$	$MgSO_4$	$NaHCO_3$	$CaCl_2$	Tris-Cl pH 7.8	Sucrose	Choline Cl	LiCl	$NaMeSO_4$	$KMeSO_4$	$Mg(EtSO_4)_2$	$Ca(EtSO_4)_2$	Tris-SO_4 pH 7.8
ASW	423	9	22.9	25.5	2.2	9.3	10	—	—	—	—	—	—	—	—
ASW, 1% Ca	423	9	22.9	25.5	2.2	0.093	10	21	—	—	—	—	—	—	—
ASW, 5% Ca	423	9	22.9	25.5	2.2	0.47	10	20	—	—	—	—	—	—	—
O Na, choline SW	—	9	22.9	25.5	—	9.3	10	—	—	—	—	—	—	—	—
O Na, choline SW, 5% Ca	—	9	22.9	25.5	—	0.47	10	20	425	425	—	—	—	—	—
O Na, lithium SW	—	9	22.9	25.5	—	9.3	10	—	—	425	—	—	—	—	—
O Na, lithium SW, 1% Ca	—	9	22.9	25.5	—	0.093	10	21	—	425	—	—	—	—	—
O Na, lithium SW, 5% Ca	—	9	22.9	25.5	—	0.47	10	20	—	425	—	—	—	—	—
O Na, Tris SW	—	9	22.9	25.5	—	9.3	645*	—	—	—	—	—	—	—	—
O Na, Tris SW, 5% Ca	—	9	22.9	25.5	—	0.47	645*	20	—	—	—	—	—	—	—
O Na, sucrose SW, (2 mol-for-mol)	—	9	22.9	25.5	—	9.3	10	850	—	—	—	—	—	—	—
O Na, sucrose SW, (2 mol-for-mol) 5% Ca	—	9	22.9	25.5	—	0.47	10	870	—	—	—	—	—	—	—
O Na, sucrose SW (isosmotic)	—	9	22.9	25.5	—	9.3	10	617	—	—	—	—	—	—	—
O Na, sucrose SW (isosmotic) 1% Ca	—	9	22.9	25.5	—	0.093	10	638	—	—	—	—	—	—	—
O Na, sucrose SW (isosmotic) 5% Ca	—	9	22.9	25.5	—	0.47	10	637	—	—	—	—	—	—	—
O K SW	423	—	22.9	25 5	2.2	9.3	—	13	—	—	—	—	—	—	—
O Cl SW	—	—	—	25.5	2.2	—	—	—	—	—	423	9	22.9	9.3	—
O Mg SW	423	9	—	—	2.2	9.3	—	—	—	—	—	—	—	—	76.5

* at pH 7.8, 2/3 of the Tris is ionized; the control ASW was made hypertonic with 215 mM Sucrose.

Methylsulfate is denoted by $MeSO_4$; ethylsulfate by $EtSO_4$.

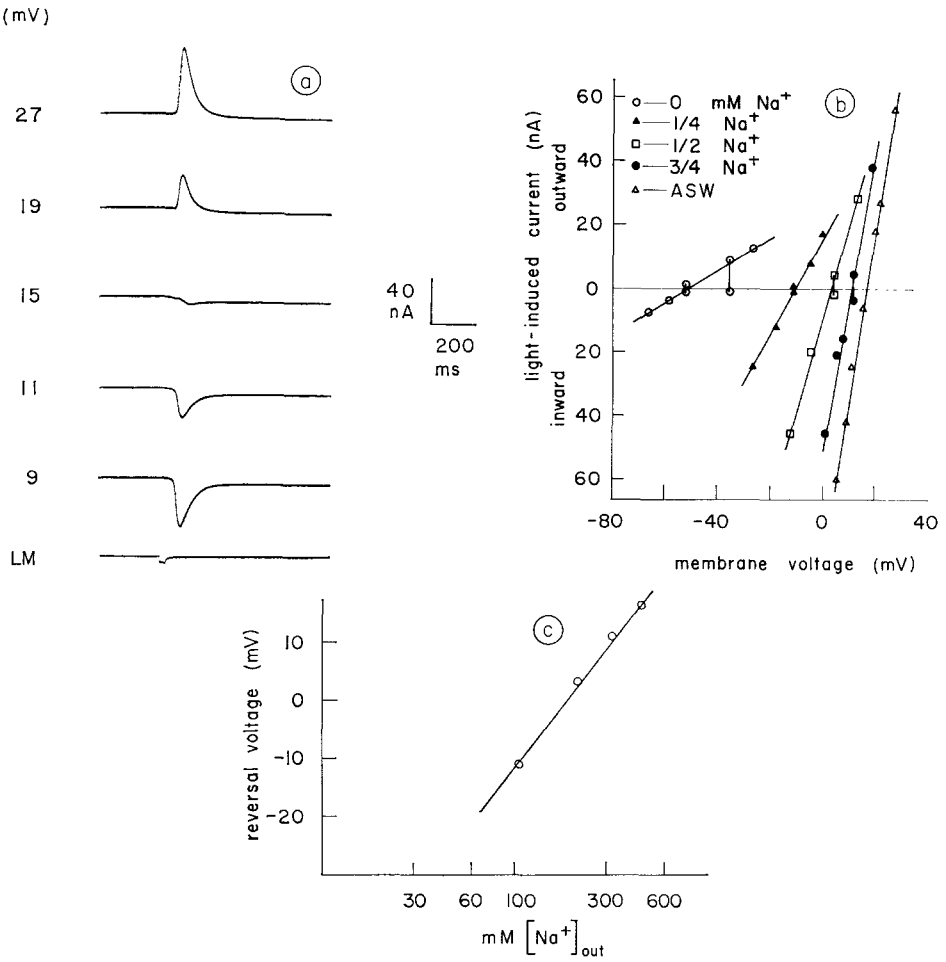

FIGURE 1. (*a*) Light-induced current at different membrane voltages. For voltages more negative than +17 mV, the currents were inward; for voltages more positive than +17 mV the current were outward. Membrane voltage during each current record is marked in the left column. Stimulus monitor is labeled LM. (*b*) Peak light-induced current measured from data as in *a* plotted vs. membrane voltage for several values of Na_o (given as the fraction of the concentration of Na in ASW) with NaCl replaced by osmotically equivalent amounts of sucrose. When biphasic responses were observed, both positive and negative peak values were plotted (symbols connected by vertical line). Data from *a* are plotted on the ASW line. (*c*) Zero light-induced current was interpolated from the data in *b*. The corresponding values of voltage, defined to be reversal voltage, (V_{rev}), were plotted vs. $\log_{10} Na_o$.

changed and V_{rev} was determined again, finally ASW was returned to the chamber and V_{rev} measured again. This procedure was repeated for each change of ion concentration.

In this paper, ion concentrations are denoted by the chemical symbols and a

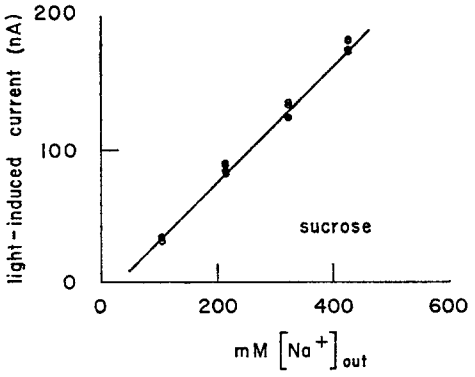

FIGURE 2. Light-induced current as a function of Na_o. Same cell as Fig. 1. Open and closed symbols represent separate sequences of measurements. Membrane voltage was clamped to resting voltage in the dark.

subscript to indicate whether intracellular or extracellular is meant. For example Na_o is used instead of $[Na^+]_{out}$.

RESULTS

The reversal voltage became more negative as the concentration of Na in the bathing solution was reduced (Fig. 1 *c*) by replacement of NaCl by sucrose, with the solutions maintained isosmotic. The mean slope of the curve measured at 425 mM Na is 51 mV/decade increase in Na_o (Table II and Fig. 3 A). Similar values for the slope were obtained when each mole of NaCl was replaced by 2 mol of sucrose, although in this latter case the osmolarity increased as Na_o decreased (Table II and Fig. 3 *A*). In addition, removal of 95% of the Ca from the solutions had no noticeable effect on the change of reversal voltage when NaCl was replaced by sucrose (Table II and Fig. 3 *a*). For all 13 cells for which NaCl was replaced by sucrose, the mean slope measured at Na_o = 425 mM was 55.2 (±10.5 SD) mV/decade increase in Na_o. That is, for Na_o between 212 and 637 mM, the slope of the V_{rev} vs. log Na_o curve was approximately 55 mV/10-fold change in Na_o even with changes in ionic strength, with small changes in osmolarity, or with reduction of Ca_o. However, for Na_o of 106 mM or less, the reversal voltage remained significantly more positive than predicted by a straight line with a slope of 55 mV/decade drawn through the points close to the normal Na_o (Fig. 3 *a*).

When sodium ions were replaced with either Tris or choline ions, reversal voltage also became more negative as Na_o was decreased. The mean slopes of the V_{rev} vs. log Na_o curves measured at 425 mM = Na_o were 53 (±5.5 SD) mV/decade for Tris replacing Na and 20 mV/decade for choline replacing Na (Table II and Fig. 3 *b*). When sodium ions were replaced by lithium ions, however, there was no measureable change in reversal voltage

TABLE II

IONIC REPLACEMENT AND REVERSAL VOLTAGE

Bath solution	Ca_o	Slope at 430 mM = Na_o
	mM	*mV/decade*
NaCl replaced (2 mol-for-mol) by sucrose	9.3	64
		41
		62 mean ≃ 56
NaCl replaced (2 mol-for-mol) by sucrose	0.47	43
		61
		52
		54
		57 mean ≃ 53
NaCl replaced by isosmotic sucrose	9.3	42
		44
		66 mean ≃ 51
NaCl replaced by isosmotic sucrose	0.093	75
		57 mean ≃ 66
All sucrose replacements	—	mean ≃ 55.1
		S.D. ≃ 10.5
Na replaced by choline	9.3	20
		18
		21 mean ≃ 20
Na replaced by choline	0.47	29
		32 mean ≃ 31
Na replaced by Tris	9.3	44
		58
		56 mean ≃ 53
Na replaced by Tris	0.47	55
		52 mean ≃ 54
All Tris replacements	—	mean ≃ 53
		SD ≃ 5.5

of the light response (Table III and Fig. 3 *b*). Removal of 95% of the Ca from the solutions had no noticeable effect on the change of reversal voltage when Na was replaced by Tris or Li. However, removal of 95% of the Ca_o led to a slightly steeper slope of the V_{rev} vs. log Na_o curve when Na was replaced by choline (Table II). Thus, the slope of the V_{rev} vs. log Na_o curve, when sodium was substituted by Tris (for Na_o between 106 and 637 mM), was approximately the same as the slope when NaCl was replaced by sucrose, but differed markedly from the slope when sodium was replaced by choline or lithium.

We also determined the amplitude of the current induced by fixed intensity stimuli, with the voltage held at dark resting potential in ASW, for various values of Na_o when NaCl was replaced by sucrose (Fig. 2). After Na_o was reduced (to not less than 212 mM), the light-induced current became smaller and reached a new steady value after about 2 min. If the Na_o was reduced to

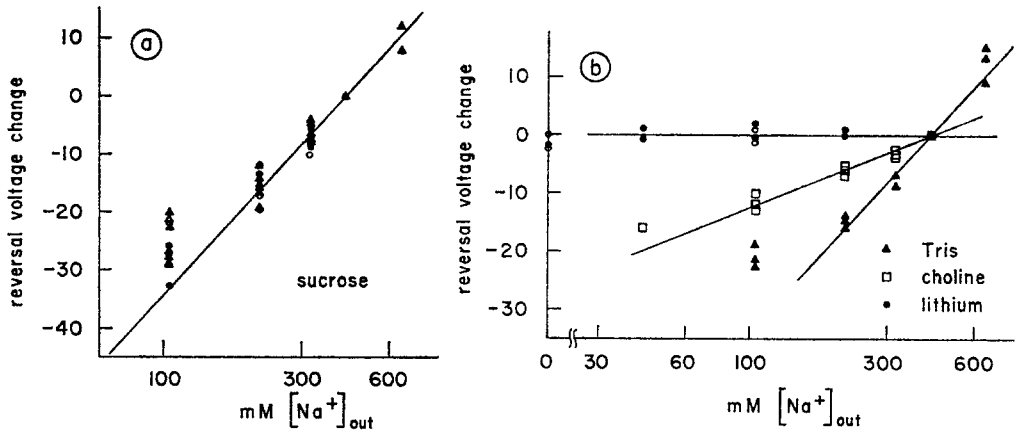

FIGURE 3. (*a*) Reversal voltage change vs. $\log_{10}$ Na_o for 13 cells. To compare data from different cells, the value of V_{rev} in ASW for each cell was subtracted from all values measured from that cell. Circles: isosmotic replacement of NaCl by sucrose. Triangles: each mole of NaCl replaced by 2 mol of sucrose. Open symbols: normal Ca_o. Closed symbols: reduced Ca_o. The line is plotted at a slope of 55 mV/decade change in Na_o. (*b*) Reversal voltage change vs. $\log_{10}$ Na_o for Tris, choline, and lithium replacing sodium. Open circles (for lithium) were recorded with 0.47 mM Ca in the bath. All other data recorded with 9.3 mM Ca in the bath.

TABLE III

IONIC REPLACEMENT AND REVERSAL VOLTAGE

Bath solution	Mean change in V_{rev}	N	Ratio LIC in test bath/ LIC in ASW
20% Cl	−3.0 mV	3	0.89
0 Cl	−3.7	4	0.92
20% Mg	−0.5	4	1.1
0 Mg	−1.4	6	1.1
20% K	−1.3	3	0.96
0 K	−1.0	3	0.93
20% Ca	−1.7	4	1.25
1% Ca	−1.0	4	1.3
0 Na, sucrose	−32	6	
0 Na, sucrose, 1% Ca	−28.8	4	
0 Na, Li, 1% Ca	−2.0	3	

The second column is the change in V_{rev} relative to V_{rev} measured in ASW. N is the number of cells. The ratio of the peak amplitude of the light-induced current (LIC) measured 2 min after changing solutions to the peak amplitude of the LIC measured in ASW is given in the fourth column.

106 mM or less, the light-induced current did not become stable until longer than 2 min.; sometimes the light response became very small, or zero, and then recovered partially (Millecchia and Mauro, 1969 *b*). Light-induced currents in Fig. 4 were measured after they reached a steady size. The relationship

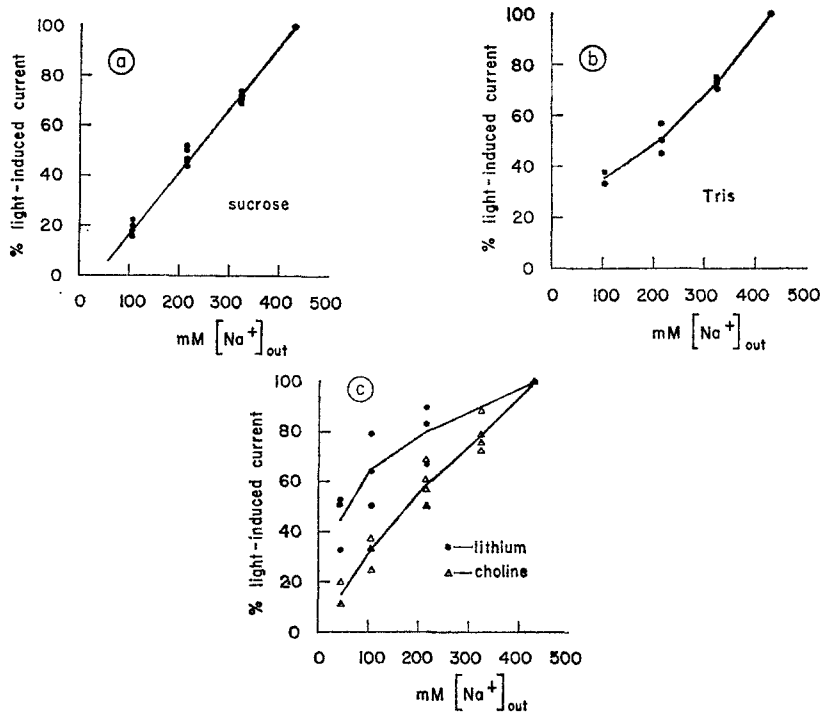

FIGURE 4. (*a*) Percent light-induced current vs. Na_o. To compare data from five cells, the peak currents for each cell were divided by the peak current recorded in ASW for that cell. NaCl was replaced by isosmotic amounts of sucrose. (*b*) Percent light-induced current vs. Na_o, with Na replaced by Tris, for three cells. (*c*) Percent light-induced current vs. Na_o, with Na replaced by either lithium (three cells) or choline (three cells).

between light-induced current and Na_o was approximately linear for Na_o above 106 mM (Fig. 4 *a*). The extrapolated light-induced current reversed sign near $Na_o = 0$. This suggested that the receptor potential might reverse sign near resting potential in sodium-free (sucrose) seawater.

For different cells bathed in sodium free (sucrose) seawater, the reversal voltage varied between −15 and −46 mV, and dark resting voltage varied between −35 and −42 mV. Usually, the reversal voltage was more positive than dark resting voltage, although occasionally, the opposite was found. For example, as shown in Fig. 5, after replacement of NaCl by sucrose, the dark resting potential initially became more negative (hyperpolarized), then gradually depolarized to a new steady value slightly more negative than dark, resting potential in ASW. Soon after the replacement, no receptor potentials were elicited; later, receptor potentials gradually reappeared, reversed in sign. After NaCl was returned to the bath, resting voltage and receptor potentials (both magnitude and sign) recovered.

The amplitude of the currents induced by stimuli of fixed intensity, with

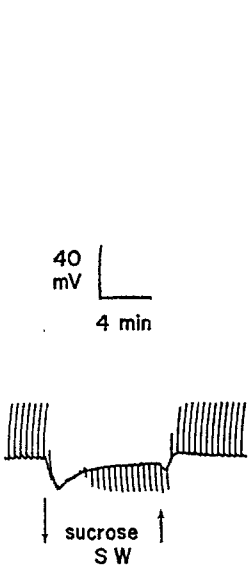

FIGURE 4

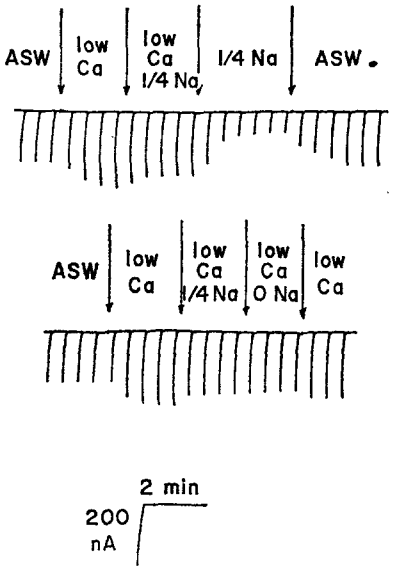

FIGURE 5

FIGURE 5. Receptor potentials elicited by repetitive flashes (50-ms duration) during replacement of each mole of NaCl with 2 mol of sucrose in the bathing solution.

FIGURE 6. Chart records of light-induced current. A 50-ms flash was given once every 30 s. The changes of bath solution are marked above each trace. Three-fourths of the sodium was replaced by lithium (= one-fourth Na solution) or all the sodium was replaced by lithium (= 0 Na solution). The replacement of sodium by lithium did not markedly reduce the size of the light-induced currents when Ca_o was also reduced (low Ca = 0.093 mM Ca).

the voltage clamped to dark, resting potential, also decreased when Na_o was replaced with Tris or choline (Fig. 4 *b* and *c*). Similarly, the light-induced currents decreased in size when Na was replaced by Li (Figs. 4 *c* and 6); however, the size of the light-induced current did not diminish rapidly (Fig. 6) when Li replaced Na in the absence of 99% of the Ca_o.

As mentioned above, the value of V_{rev} in solutions containing less than 212 mM Na was significantly more positive than would be predicted from the slope of the V_{rev} vs. log Na_o curve measured at Na_o = 425 mM. This might occur if significant inward current were carried by another cation (e.g., Ca) when Na_o was reduced to low values. This prompted us to examine the effect of Ca_o on V_{rev} when the cells were bathed in Na-free, sucrose SW. For four cells, the reversal voltage changed an average of about 3 mV when 99% of the $CaCl_2$ was replaced by sucrose and no Na was present in the bath (Table III). Moreover, reduction of Ca_o in the absence of extracellular Na led to an increase in amplitude of currents induced by stimuli of fixed intensity. Thus, it is unlikely that Ca carries a significant fraction of the light-induced current measured from cells bathed in Na-free (sucrose) seawater.

As previously reported by Millecchia and Mauro (1969 *b*), in the presence of the normal concentration of Na in ASW, removal of Ca_o leads to an increase in size of the light-induced current at a fixed voltage (see also Lisman and Brown, 1972). Millecchia and Mauro (1969 *a*) reported that changes of Mg_o, K_o, or Cl_o did not affect receptor potentials; however, we find that removing all or part of the Mg_o increases the size of the light-induced current, and reducing either K_o or Cl_o decreases the light-induced current (Table III). In these experiments either (*a*) KCl or $CaCl_2$ was replaced by sucrose, (*b*) $MgCl_2$ and $MgSO_4$ were replaced by sucrose or Tris-Cl, or (*c*) chloride ion was replaced by methylsulfate and ethylsulfate ions. We have confirmed (Millecchia and Mauro, 1969 *b*) that with the normal concentration of Na in ASW, reducing the concentration of Mg, Ca, Cl, or K does not noticeably alter the reversal voltage of the light response (Table III).

DISCUSSION

In this study, we used repetitive stimuli to maintain the cell in a constant adaptational state. The stimuli were brief; thus, we minimized the component of the response due to the slowly changing light-induced process described by Lisman and Brown (1971). The responses measured were directly comparable to the rapidly changing component of Lisman and Brown (1971) and the light-induced currents reported by Millecchia and Mauro (1969 *b*).

If the rapidly changing component of the light response were generated solely by a change of the sodium conductance, then a simple electrodiffusion model would predict that reversal voltage (V_{rev}) would be equal to the Nernst potential for sodium (E_{Na}):

$$V_{rev} = E_{Na} = \frac{RT}{F} \cdot ln \frac{[A_{Na}]_{out}}{[A_{Na}]_{in}},$$

where $[A_{Na}]$ is the activity of sodium in the solution. The activity can be represented

$$[A_{Na}] = \gamma_{Na} \cdot Na,$$

where γ is the activity coefficient and Na is the analytical concentration. Therefore

$$V_{rev} = \frac{RT}{F} \cdot \left[ln \frac{\gamma_{out}}{\gamma_{in}} + ln \frac{Na_o}{Na_i} \right].$$

When monovalent cations are used to replace sodium ions, the ionic strength of the solutions should remain approximately constant. In the absence of data on the intracellular activity coefficient for monovalent cations in *Limulus* photoreceptors, we will assume the ratio $\gamma_{out}/\gamma_{in} = 1$, and that γ_{in} doesn't

change when extracellular sodium is replaced by a monovalent cation. Thus the slope of a graph of V_{rev} vs. $\log_{10} Na_o$ is predicted to be $RT/F \times 2.3 \simeq 58$ mV per 10-fold change of Na_o. However, when NaCl is replaced by sucrose, the ionic strength of the solutions is reduced. In these solutions, γ_{out} should become larger. Using Davies' modification of the extended Debye-Hückel law (Robinson and Stokes, 1959) $RT/F \cdot (\ln \gamma_{out}/\gamma_{in}) \simeq +1$ mV for replacement of 90% of the NaCl by sucrose, assuming γ_{in} does not change. That is, the slope of V_{rev} vs. Na_o is predicted to be approximately 57 mV/decade for replacement of NaCl by sucrose. Assuming that the reference electrode doesn't introduce a significant voltage when the solutions are changed, we determined that, for values of Na_o close to the normal value in ASW, the mean slope for 13 cells was 55.2 $\pm$ 10.5 SD mV/decade (for NaCl replaced by sucrose) and the mean slope for five cells was 53 $\pm$ 5.5 SD mV/decade (for Na replaced by Tris).

V_{rev} is more positive than predicted by the Nernst relation when Na_o is small; this might arise for at least three reasons. First, if Na_i were decreased by decreases in Na_o during the time necessary to determine the reversal voltage, then E_{Na} would become relatively more positive; we have no independent measure of intracellular sodium concentration. Second, the ion used to replace Na might partially substitute for Na in the light-induced conductance mechanism. Third, one or more ions other than Na might participate in the generation of the light response.

When Na is replaced by either Li or choline, behavior of V_{rev} vs. Na_o is not predicted by the Nernst equation. One can interpret this to mean that these ions can substitute for Na either partially or totally, during the light-induced change in membrane permeability. Following the treatment of Chandler and Meves (1965), the effective equilibrium voltage (V_{eff}) for the light response should be

$$V_{rev} = V_{eff} = \frac{RT}{F} \cdot ln \left[\frac{\Delta P_{Na} \cdot Na_o + \Delta P_Y \cdot Y_o}{\Delta P_{Na} \cdot Na_i + \Delta P_Y \cdot Y_i} \right],$$

where Y is either lithium or choline and $Na_o + Y_o = 425$ mM. Assuming that the permeability changes (ΔP's) are not dependent on ion concentrations, that Na_i doesn't change for small changes in Na_o and that during the time of the measurement $\Delta P_Y \cdot Y_i$ is negligible, we can calculate the ratio of the permeability changes $\Delta P_Y/\Delta P_{Na}$ knowing the reversal voltages for each of two concentrations of Y_o.

$$V_{rev,1} - V_{rev,2} = \frac{RT}{F} \cdot ln \left[\frac{Na_{o,1} + \frac{\Delta P_Y}{\Delta P_{Na}} \cdot Y_{o,1}}{Na_{o,2} + \frac{\Delta P_Y}{\Delta P_{Na}} \cdot Y_{o,2}} \right].$$

From the data in Fig. 3 *b*, the ratios are $\Delta P_{Li}/\Delta P_{Na} \simeq 1.0$ and $\Delta P_{Chol}/\Delta P_{Na} \simeq 0.5$. As also seen in Fig. 3 *b*, the plot of V_{rev} vs. log Na_o deviates from the Nernst prediction when Na is replaced by Tris. Using the above equation, and the data at Na_o = 106 mM, $\Delta P_{Tris}/\Delta P_{Na} \simeq 0.2$. However, the behavior of V_{rev} vs. log Na_o, when sodium is replaced by Tris, is nearly the same as that when sodium is replaced by sucrose (for Na between 106 mM and 637 mM; Fig. 3 *a* and *b*). The simple application of the above equation for two ions probably is not adequate; the ratio $\Delta P_{Tris}/\Delta P_{Na}$ may be much smaller than the calculation indicates. Thus, although light normally induces an increase in permeability to sodium ions, either lithium or choline can participate (at least partially) in the generation of receptor responses; this has also been found in other receptors (Obara and Grundfest, 1968; Obara, 1968).

When sodium ions are replaced by either Tris or sucrose, V_{rev} deviates from the prediction of the Nernst equation for lower values of Na_o. This might indicate that light normally induces a change in membrane permeability to an ion (or ions) other than Na. There is evidence which might indicate that light may induce an increase in permeability to K in *Limulus* ventral photoreceptors. Holt and Brown (1972) found an increase in unidirectional ^{42}K efflux elicited by illumination of a ventral eye. In the same eye, they measured ^{42}K efflux and membrane voltage simultaneously. Using the measured values of depolarizing receptor potentials and the flux equation with the constant-field assumption, they calculated the ^{42}K efflux was larger than that expected if the membrane permeability to potassium were independent of both light and voltage. Holt and Brown could not decide between these possibilities from their ^{42}K efflux data. We have found additional evidence supporting a light-induced increase in potassium permeability. With Na_o = 425 mM, there was a small decrease in light-induced current when K_o was reduced. Also, again following the treatment of Chandler and Meves (1965) and Hille (1972) for the difference in reversal voltage (ΔV_{rev}) measured in two solutions:

$$V_{rev,1} - V_{rev,2} = \Delta V_{rev} = \frac{RT}{F} \cdot \left[ln \left[\frac{Na_{o,1} + \frac{\Delta P_K}{\Delta P_{Na}} \cdot K_{o,1}}{Na_{o,2} + \frac{\Delta P_K}{\Delta P_{Na}} \cdot K_{o,2}} \right] + ln \frac{\gamma_{out,1}}{\gamma_{out,2}} \right].$$

In principle, this equation could be used to calculate $\Delta P_K/\Delta P_{Na}$ from data such as in Fig. 3 *a*. However, with K_o = 9 mM and Na_o greater than 100 mM, small changes in ΔV_{rev} lead to large changes in the calculated values of $\Delta P_K/\Delta P_{Na}$. For example, for a change in Na_o from 425 mM to 212 mM, with K_o = 9 mM, $\Delta V_{rev} = -15.3$ mV ± 2.6 mV SD (n = 13). For $\Delta V_{rev} = -15.3$ mV, $\Delta P_K/\Delta P_{Na} \simeq 3.9$ whereas the Nernst equation predicts $\Delta V_{rev} = -17.2$ mV, $\Delta P_K/\Delta P_{Na} = 0$. The variability of the data (e.g. Fig. 3 *a*) is too large to allow a reliable calculation of $\Delta P_K/\Delta P_{Na}$. The calculation of

$\Delta P_K/\Delta P_{Na}$ would be less sensitive to small changes in ΔV_{rev} if K_o were made larger and Na_o were decreased.

To test this argument, we have done the following preliminary experiment. With Na_o = 212 mM, K_o was changed from 9 mM to 220 mM by replacing sucrose with KCl. The mean reversal voltage (n = 4) was 8.6 mV more positive in the solution having higher K_o. From this value for ΔV_{rev}, we calculate that $\Delta P_K/\Delta P_{Na} \simeq 0.5$. We consider this result to be tentative since the experiments were not done with a constant K × Cl product and changes in osmolarity can lead to small changes in V_{rev} (J. E. Brown, unpublished observation). With a ratio of the change in permeability $\Delta P_K/\Delta P_{Na}$ = 0.5, the plot of V_{rev} vs. log Na_o would deviate from the line predicted by the Nernst equation in the same direction as do the data in Fig. 3 *a*, although less than does the mean value for V_{rev} at Na_o = 106 mM. Also, V_{rev} would change by less than 1 mV when K_o was reduced from 9 mM to 0.09 mM with Na = 425 mM, which agrees with the failure to measure a change in V_{rev} in these solutions. Thus, it seems probable that the ratio of $\Delta P_K/\Delta P_{Na}$ is not insignificant; that is, light induces a change in membrane permeability to potassium as well as to sodium.

Although V_{rev} does not change noticeably when Na is replaced by Li the size of the current induced by a fixed size stimulus is attenuated. This attenuation tends not to appear when Ca_o is reduced. We suggest that lithium ions can substitute for Na during the light-induced change in membrane conductance, but do not behave identically in the mechanisms which regulate the intracellular concentrations of Na and Ca (cf. Lisman and Brown, 1972). That is, Li may enter the cell during the light response, but unlike Na may not be rapidly removed. Lisman and Brown (1972) proposed that an increase in Na_i lead to an increase in Ca_i. If Li also mimics Na in this action, then an increase in Li inside the cell may cause the Ca_i to rise. This rise in Ca_i then might signal a reduction in the size of the light-induced conductance change (Lisman and Brown, 1972).

In contrast to our results, H. M. Brown et al. (1970) found that for the photoreceptors of *Balanus eburneus*, the slope of graph of reversal voltage for the light response as a function of Na_o was 10–15 mV/decade in normal Ca concentrations and was 16–21 mV/decade for changing Na_o in the absence of Ca_o, replacing sodium with either Tris or sucrose. They also found that the slope of V_{rev} vs. Ca_o was 15–20 mV/decade in the absence of Na_o. Thus *Limulus* and barnacle photoreceptors differ with regard to the behavior of V_{rev} when challenged with changes in the concentrations of extracellular cations.

In summary, the light response of ventral photoreceptors of *Limulus* appears to arise from a light-induced increase in membrane permeability. At the normal value of extracellular potassium, the slope of the plot of V_{rev} vs. log Na_o approaches that predicted by the Nernst equation for sodium, for high

values of Na_o. Nevertheless, light probably induces an increase in membrane permeability to both sodium and potassium ions.

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BIBLIOGRAPHY

BROWN, J. E., and M. I. MOTE. 1971. Na^+-dependence of reversal of light-induced current in *Limulus* ventral photoreceptors. *Biol. Bull.* (*Woods Hole*). **141**:379.

BROWN, H. M., S. HAGIWARA, A. KOIKE, and R. MEECH. 1970. Membrane properties of a barnacle photoreceptor examined by the voltage-clamp technique. *J. Physiol.* (*Lond.*). **208**:385.

CLARK, A. W., R. MILLECCHIA, and A. MAURO. 1969. The ventral photoreceptor cells of *Limulus*. I. The microanatomy. *J. Gen. Physiol.* **54**:289.

CHANDLER, K., and H. MEVES. 1965. Voltage clamp experiments on internally perfused giant axons. *J. Physiol.* (*Lond.*). **180**:788.

DEMOLL, R. 1914. Die Augen von *Limulus*. *Zool. Jahrb. Abt. Anat. Ontog. Tiere.* **38**:443.

LISMAN, J. E., and J. E. BROWN. 1971. Two light-induced processes in the photoreceptor cells of *Limulus* ventral eye. *J. Gen. Physiol.* **58**:544.

LISMAN, J. E., and J. E. BROWN. 1972. The effects of intracellular iontophoretic injection of calcium and sodium ions on the light response of *Limulus* ventral photoreceptors. *J. Gen. Physiol.* **59**:701.

HILLE, B. 1972. The permeability of the sodium channel to metal cations in myelinated nerve. *J. Gen. Physiol.* **59**:637.

HOLT, C. E., and J. E. BROWN. 1972. Ion fluxes in photoreception in *Limulus* ventral eye. I. The potassium light-response. *Biochim. Biophys. Acta.* **274**:140

MILLECCHIA, R., and A. MAURO. 1969 *a*. The ventral photoreceptor cells of *Limulus*. II. The basic photoresponse. *J. Gen. Physiol.* **54**:310.

MILLECCHIA, R., and A. MAURO. 1969 *b*. The ventral photoreceptor cells of *Limulus*. III. A voltage-clamp study. *J. Gen. Physiol.* **54**:331.

OBARA, S. 1968. Effects of some organic cations on generator potential of crayfish stretch receptor. *J. Gen. Physiol.* **52**:363.

OBARA, S., and H. GRUNDFEST. 1968. Effects of lithium on different membrane components in crayfish stretch receptor. *J. Gen. Phsyiol.* **52**:635.

ROBINSON, R. A., and R. H. STOKES. 1959. *Electrolyte Solutions*. Butterworth & Co. (Publishers) Ltd., London. 232.