Cation and Anion Sequences in Dark-Adapted *Balanus* Photoreceptor

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ABSTRACT Anion and cation permeabilities in dark-adapted *Balanus* photoreceptors were determined by comparing changes in the membrane potential in response to replacement of the dominant anion (Cl^-) or cation (Na^+) by test anions or cations in the superfusing solution. The anion permeability sequence obtained was $P_I > P_{SO_4} > P_{Br} > P_{Cl} > P_{isethionate} > P_{methanesulfonate}$. Gluconate, glucuronate, and glutamate generally appeared more permeable and propionate less permeable than Cl^-. The alkali-metal cation permeability sequence obtained was $P_K > P_{Rb} > P_{Cs} > P_{Na} \approx P_{Li}$. This corresponds to Eisenman's sequence IV which is the same sequence that has been obtained for other classes of nerve cells in the resting state. The values obtained for the permeability ratios of the alkali-metal cations are considered to be minimal. The membrane conductance measured by passing inward current pulses in the different test cations followed the sequence, $G_K > G_{Rb} > G_{Cs} > G_{Na} > G_{Li}$. The conductance ratios obtained for a full substitution of the test cation agreed quite well with permeability ratios for all the alkali-metal cations except K^+ which was generally higher.

INTRODUCTION

There has been considerable interest in characterizing biological membranes as to the most probable mechanism(s) of ion permeation. One approach to the problem has arisen from studies of the mechanism of ion selectivity in nonbiological systems, such as glasses of various composition (Eisenman, 1962, 1967; Eisenman and Conti, 1965). These systems have been well characterized since the nature of the moieties which mediate ion permeation is known. Advantage can be taken of these systems by comparing their behavior to an unknown biological system to see what features are the same or different, so that an appropriate model of ion permeation can be developed for the unknown system. Unfortunately, not all of the diagnostic techniques that are readily applicable to nonbiological membranes can be applied to the study of smaller living cells because of the difficulty of altering the internal ionic environment of the cell. Thus, the study of bi-ionic potentials and symmetrical solution dilution potentials are necessarily precluded. However, the study of ion selectivity derived from changes in membrane potential and membrane conductance in different ionic media may provide useful information concerning the permeability mechanism involved. Thus, coincidence or noncoincidence of the so-called conductance and permeability sequence is thought to depend on the mode of ion

permeation. Eisenman (1967) observed that a certain glass formulation constituting a negative fixed charge membrane had a Na^+-K^+ permeability ratio that was the obverse of the conductance ratio. The permeability ratio (P_x/P_y) for two ions x and y and can be expressed as:

$$\frac{P_x}{P_y} = \mu_x/\mu_y \cdot \beta_x/\beta_y$$

where μ is the mobility of the ion in the membrane and β represents the partition coefficient of the ion between the solution and the membrane. The ratio β_x/β_y is equivalent to the binding constant ratio of a membrane site for a cation pair (K_{xy}). Eisenman rationalized the above observation on the basis that the most permeable ion was not the most mobile, and that the mobility ratio was much closer to unity than the binding constant ratio. Similar results have been obtained for anion permeability and conductance in *Balanus* muscle fibers and as a consequence it was suggested that permeation might proceed via a fixed positively charged site (Hagiwara et al., 1971). Coincidence of the conductance and permeability sequences might be considered prima facie evidence for a neutral site or neutral carrier mechanism of ion permeation. Such behavior has been reported for certain artificial and biological membrane systems (Eisenman et al., 1968; Barry et al., 1971).

The present paper presents results from the dark-adapted barnacle photoreceptor membrane in which anion and cation selectivity sequences were evaluated from changes in membrane potential in different test solutions. Membrane conductance was also measured to provide another index of the selectivity sequence. The cation conductance and permeability sequences were found to be the same by either technique and the permeability and conductance ratios were in quite good agreement. The anion sequence followed the free solution mobility sequence of the halide anions. A preliminary report of some of the work has appeared previously (Saunders and Brown, 1974).

MATERIALS AND METHODS

The procedure for recording from barnacle photoreceptors has been described previously (Brown et al., 1970). Briefly, the method consisted of removal of the lateral ocellus from the barnacle and disection of the pigment epithelium and the tapetum to expose the three photoreceptor cells. One of the photoreceptors was impaled with one or two 3 M KCl-filled micropipettes (5–10 MΩ resistance). Membrane potential was recorded differentially between one internal electrode and a similar extracellular electrode. The steady-state resting potential of these cells was generally between −40 and −50 mV after a period of at least 15 min of dark adaptation. A second intracellular electrode was used in some experiments to pass current across the cell membrane. Current was measured with an operational amplifier in the current-to-voltage configuration.

Cation test solutions were made up to the same ionic strength and pH as those of normal barnacle saline (Brown et al. 1970). All solutions contained the normal complement of divalent cations, and mixtures of the various salines were made to give families of solutions in which the univalent control alkali-metal cation (Na^+) was systematically replaced by the test cation. Sodium was the control cation in most experiments, so that the preparations were superfused with solutions of $[Na^+] + [K^+] = 470$ mM, $[Na^+] + [Rb^+] = 470$ mM, etc.

An electrogenic Na^+-K^+ pump has been proposed (Koike et al., 1971) to explain the significant changes in membrane potential after termination of illumination, or after K^+'s removal from or return to the bath. Several different procedures were used to evaluate the effect of an electrogenic pump on cation selectivity sequences. Preparations were studied in the dark and control solutions used between applications of the test cations were always K^+-free. In some experiments, Li^+ was used as the control cation since it has been shown that Li^+ reduces the effect of the electrogenic pump (Koike et al., 1971). In other experiments, preparations were pretreated with 10^{-5} M ouabain.

RESULTS

Alkali-Metal Permeability Sequence

Fig. 1 illustrates changes in the membrane potential as K^+-free NaCl barnacle

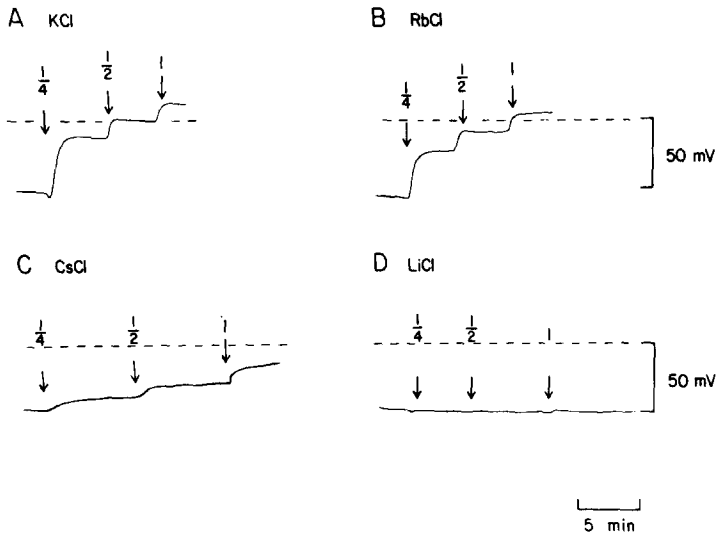

FIGURE 1. Changes in membrane potential in a dark-adapted *Balanus* photoreceptor as K^+-free 470 mM NaCl barnacle saline was replaced by different amounts of KCl, RbCl, CsCl, or LiCl salines in panels A, B, C, and D, respectively. All salines contained the normal complement of Ca^{++} and Mg^{++}. The reference potential is indicated by the dashed line.

saline was systematically replaced by KCl, RbCl, CsCl, and LiCl. In panel A, the preparation was successively superfused with Na^+-K^+ barnacle salines. The resting membrane potential of this dark-adapted photoreceptor was −51 mV in K^+-free, NaCl barnacle saline at the beginning of the experiment. Replacement of 1/4 of the NaCl with an equimolar quantity of KCl resulted in a membrane depolarization to −14 mV. Replacement of 1/2 of the Na^+ by K^+ depolarized the membrane to −2 mV, and when all of the NaCl was replaced by KCl, the membrane potential was +10 mV. The remaining panels show changes in membrane potential as Rb^+, Cs^+, and Li^+ replaced Na^+. In this cell the membrane was slightly hyperpolarized as Li^+ replaced Na^+. At the end of the experiment and 27 solution changes, the membrane potential was −50 mV.

Photoreceptors were always dark-adapted at least 15 min before the experiments began. Sometimes there was a slight depolarization when the NBS was replaced with K^+-free saline. A small initial hyperpolarization was often observed when K^+ was returned to the superfusate as shown in Fig. 1 ($^1/_4$ KCl), but this was not a constant feature of all the experiments. The initial hyperpolarization probably represents K^+-activation of an electrogenic pump (Koike et al., 1971). Some preparations also showed this behavior when K^+-free, NaCl saline was replaced by $^1/_4$ Rb^+ saline. Although an electrogenic pump may be activated when K^+ or Rb^+ is added to the NaCl barnacle saline, there should be little error in the determination of the membrane potential in these salines since the depolarization is associated with an increase in membrane conductance which should minimize the contribution of an electrogenic pump to the membrane potential under these conditions.

Chloride-Free and Ouabain-Containing Salines

Several experiments were conducted to determine whether or not Cl-free saline or ouabain would affect results obtained when Na^+ was replaced by a test cation. Membrane potential changes were observed as K^+ replaced Na^+ in Cl^- and $MeSO_3^-$ solutions. Each test saline was preceded by exposure to NBS containing 8 mM K. In each of several different cells the potential change in Cl-free saline was always somewhat less for a given K^+ concentration change than it was in the Cl-containing solutions (not illustrated). For this reason, subsequent studies were conducted with Cl-containing solutions. Ion replacement studies were also conducted in ouabain since the membrane current associated with an electrogenic pump might alter potential changes to different test cations. In these experiments the cells were pretreated with 10^{-5} M ouabain. The ouabain was considered to be effective when a 1-s test light flash was not followed by a membrane hyperpolarization, which is a sensitive indicator of electrogenic pump activity (Koike et al., 1971). This usually required ~20–30 min in the dark. The resting potential of cells treated in this way was usually reduced by ~5 mV. After this period, NBS was replaced by the NaCl control saline and shortly thereafter the test cation solutions were introduced to the bath. The potential changes observed with test cation substitution after treatment with ouabain were about the same as those obtained in Cl-containing salines containing no ouabain. Ouabain was not routinely used in the test cation solutions.

Chloride-Containing Salines

Fig. 2 shows data obtained from a cell studied in chloride-containing salines. These data are from a cell different from that in Fig. 1. The membrane potential is plotted as a function of the extracellular test cation concentration. The preparation was dark-adapted for 30 min before application of the test salines. After each test saline the preparation was exposed to NBS for several minutes and then to the control saline containing 470 mM NaCl before the next text cation was applied. The vertical bars at the left indicate the range of membrane potential values in the control saline (470 mM NaCl) before application of a particular test cation. The experiment was conducted by exposing the cell to each of the four test cations at a fixed concentration in the order given by the

vertical bars (left to right). The experiment proceeded from low to high concentrations of the test cations. The response of this cell was somewhat unusual in that it depolarized more to Rb^+ than to K^+ at the lower concentrations. However, a full substitution of the test cation produced a more positive E_m in K^+ than in Rb^+. The cell is typical in the sense that potential changes in Rb^+ were very close to those produced in K^+ solutions. The response to different Cs^+ concentrations was always clearly less than that to K^+ or Rb^+. Li^+ was difficult to distinguish from Na^+ except at high concentrations of Li^+; most often a full substitution of Na^+ by Li^+ produced a small membrane hyperpolarization. The permeability sequence was obtained for this cell by plotting the antilog of $E_m/25$ as a function of test cation concentration. The series of points thus obtained was

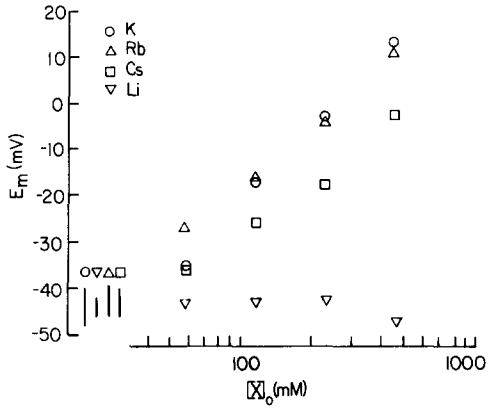

FIGURE 2. The relation between membrane potential and test cation concentration; different cell from that in Fig. 1. For the evaluation of each test cation at a fixed concentration, the preparation was first exposed to NBS and then to the control NaCl solution. The range of resting potential values in the control saline before the test solutions is represented by the bars and a corresponding symbol at the left of the x-ordinate.

fit by a line from a least-squares regression analysis and the permeability ratio P_{Na}/P_X was calculated from the slope and intercept of this relation. The permeability ratios calculated from these data were $P_{Na}/P_K = 0.078$, $P_{Na}/P_{Rb} = 0.12$, $P_{Na}/P_{Cs} = 0.21$, and $P_{Na}/P_{Li} = 1.2$. Due to scatter in the intercepts from such plots resulting from changes in the resting potential in the control saline, the data from different cells were analyzed by taking the membrane potential difference (ΔV) between one test cation concentration and the other that replaced it. For the present experiments, the use of the Goldman-Hodgkin-Katz (GHK) equation requires some simplifying assumptions: (*a*) the mean molar activity coefficients of the various ions are sufficiently similar that no serious error is introduced by using ion concentrations rather than activities; (*b*) the internal ion concentrations are not changed as the external solutions are changed. When one allows these assumptions, the terms for the internal concentrations cancel out. Since $E_m = E_{Cl}$ in *Balanus* (Brown, 1976), the P_{Cl}/P_K terms were also dropped from the equation (Mullins and Noda, 1963). Since Na^+ is always replaced by an

equimolar amount of the test cation, the total alkali-metal cation concentration is constant (M). With these simplifications, the GHK equation can be rearranged to give:

$$\exp(\Delta V/25) = \frac{1 - P_{Na}/P_K}{(P_{Na}/P_K) \cdot M} \cdot [K]_0 + 1. \quad (1)$$

A permeability ratio of control to test cation can be obtained from the slope of the relation between the left-hand term and the test alkali-metal cation concentration. If the slope is b and M is the total alkali-metal cation concentration, then:

$$P_{Na}/P_K = \frac{1}{bM + 1}. \quad (2)$$

This treatment was applied to the data obtained from several different cells run according to the protocol described above for Fig. 1. To obtain representative values for the permeability ratios, the mean value of exp $\Delta V/25$ for each of the test cation concentrations is shown for four cells in which it was possible to obtain data at all cation concentrations without appreciable changes in the resting potential of the cells. The results are shown plotted in Fig. 3; bars represent the standard errors of the mean. The lines were drawn with the constraint that exp $\Delta V/25 = 1$ at $[X]_0 = 0$.

The permeability ratios obtained from the ratios of the slopes were:

$$\begin{array}{ccccccccc} & 10.4 & & 9.1 & & 3.2 & & 1.0 \quad 1.0 & \\ P_K & > & P_{Rb} & > & P_{Cs} & > & P_{Na} & \approx & P_{Li}. \end{array}$$

The dark-adapted photoreceptor permeability sequence corresponds to Eisenman's sequence IV: $P_K > P_{Rb} > P_{Cs} > P_{Na} > P_{Li}$. Sequence III was observed in two cases: $P_{Rb} > P_K > P_{Cs} > P_{Na} > P_{Li}$. Some cells were run against lithium controls; the results were similar, i.e. they showed sequence IV, but the ratios were more variable.

Alkali-Metal Conductance Sequence

Membrane conductance was measured in different concentrations of the alkali-metal cations by passing sufficient inward current across the membrane to produce a 10-mV membrane protential change. It was difficult to obtain data for all concentrations of the different cations in the same cell because the experiments were long and changes invariably occurred in the values of E_m and G_m in the control solutions (NBS and K-free Na^+ saline). For this reason experiments were conducted to compare all cations at a single concentration in the same cell before proceeding to other concentrations. Each test solution was followed by exposure to NBS and then to the control K-free Na^+ saline so that the relative conductance ($G_{test}/G_{control}$) for different ions at the same concentration could be obtained in the same cell. The experiment was discontinued when E_m or G_m changed by more than 10% in the control salines. When the conductances in different cells were normalized by using values of relative conductance, the results compared favorably with one cell where it was possible to obtain a

complete set (48) of solution changes. Results from this cell are shown in Fig. 4 by solid symbols; different symbols represent other cells.

The conductances for K^+ and Rb^+ appeared to be quite linear over part of their range, but for a full substitution of the test cation for Na^+, the relative conductance was less than would be expected from a linear relation. This is especially evident in the cell denoted by ⊙ that was exposed to intermediate concentrations. The relative conductance in Cs^+ and Li^+ salines changed very little at lower concentrations, but upon full substitution of Cs^+ for Na^+ the conductance ratio doubled in one cell and trebled in two others. A full substitution of Li^+ produced a 20% decrease in relative conductance for one of the cells while the other showed almost no change. The data represented by solid symbols in this figure

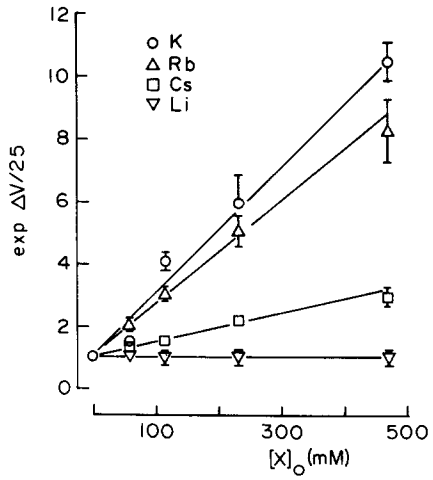

FIGURE 3. The mean of exp ΔV/25 from four cells at each test cation concentration as Na^+ in the external saline was replaced by K^+ (○), Rb^+ (△), Cs^+ (□), or Li^+ (▽). This is a graphic representation of Eq. (1). Solid lines were drawn with the constraint that at $[X]_0 = 0$, exp ΔV/25 = 1. Vertical bars = SEM.

allowed a direct comparison of the conductance and permeability ratios in a single cell. The conductance ratios with respect to Li^+ for a full substitution of the test cation were:

$$\begin{array}{ccccccccc} 19 & & 6.5 & & 2 & & 1 & & 1 \\ K & > & Rb & > & Cs & > & Na & \approx & Li. \end{array}$$

The permeability ratios obtained for the same sequence were 15.4, 9.83, 5.82, 1.2, 1.0. The mean values of the conductance ratios for a full substitution of test cation (solid lines) provide values of

$$\begin{array}{ccccccccc} 21.1 & & 8.3 & & 3.3 & & 1.11 & & 1.0 \\ K & > & Rb & > & Cs & > & Na & > & Li. \end{array}$$

These values are in quite good agreement with the mean permeability ratios obtained from Fig. 3, with the exception of K^+ where the conductance ratio is about twice as great as the permeability ratio.

Anion Permeability Sequence

If the membrane is significantly permeable to Cl^-, then partial replacement of extracellular chloride by a less permeant anion would be expected to result in a

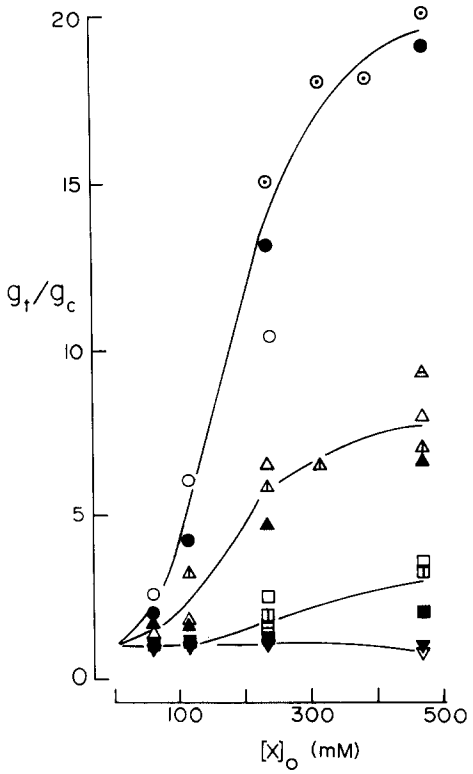

FIGURE 4. Ratios of membrane conductance in the test salines (G_t) to the preceding membrane conductance in the all-Na^+ control saline (G_c). The cell represented by (●) is the same cell as shown in Fig. 2. Different ions are represented by the same type of symbol as shown in Fig. 3. The lines were drawn by eye to approximate an average relative conductance for each cation species. For any given cell the relation appeared sigmoid in K^+ and Rb^+ salines.

depolarization of the photoreceptor membrane, while a membrane hyperpolarization would follow substitution by a more permeant anion. The relative permeabilities of several anions were determined by the method of partial chloride substitution; the divalent cations remained as chlorides. The membrane potential changes after anion substitution were small. For example, substitutions of I^- for Cl^- usually produced a potential change of −6 mV; when $MeSO_3$ was substituted for Cl^- the average depolarization was 4 mV. The values of the membrane potential changes in the test anion solutions are shown at the top of

Table I for cell 1. Estimates of the error introduced by the junction potential changes of the reference electrode were obtained at the end of each experiment for each of the ions. In the case of iodide, bromide, and methanesulfonate substitutions, the junctional potential was also calculated. In the case of methanesulfonate, the data of McBain and co-workers (1939) for the limiting conductivity of potassium methanesulfonate were taken and the limiting conductivity of the sodium salt was calculated from Kohlraush's law of the independent migration of ions. From this the mobility of the methanesulfonate ion was calculated, and in turn the expected junction potential change of the extracellular electrode was calculated from the Henderson-Planck equation. The calculated change in reference electrode junction potential for a full substitution of methanesulfonate for chloride was −1 mV. In a study of *Balanus* muscle fibers, Hagiwara et al. (1971) measured junction potential changes for I^-

TABLE I

ANION PERMEABILITY SEQUENCE BASED UPON MEMBRANE POTENTIAL CHANGES WHEN A TEST ANION WAS SUBSTITUTED FOR Cl^-

V_t-V_{Cl}	−10	−9	−6.5	−5.5	−3.5	−2	0	+2	+4.5	+5
Cell										
1	glucon	SO_4	I	glut	Br	glucur	Cl	Ise	$MeSO_3$	prop
2			I SO_4	glut	Br	glucur	Cl			
3			I SO_4	glut	Br		Cl prop	Ise	$MeSO_3$	
4			I SO_4		Br		glucon Cl glucur glut	Ise	$MeSO_3$	prop
5	glucon			glut		glucur	prop Cl	Ise	$MeSO_3$	
6	glucon			glut			Cl			
7*					Br		Cl			
8*					Br		Cl			
9*					Br		Cl			
Summary	glucon >		I > SO_4 > glut		> Br >	glucur >	Cl >	Ise >	$MeSO_3$ >	prop

* Preparation was irreversibly depolarized by F^-.

glucon = gluconate; glucur = glucuronate; glut = monobasic glutamate; ise = isethionate; prop = propionate.

and Br^- and reported +0.5 mV for both calculated and measured values; no value was given for methanesulfonate. Thus, for iodide, bromide, and methanesulfonate the change in reference electrode potential, after inversion by the differential amplifier, had the same sign as the observed membrane potential change so that the change in membrane potential could be overestimated unless some method was used to correct the experimental data. With these corrections for junction potential changes, confidence could be placed in the halide anion permeability sequence obtained; the same corrections are difficult to apply to the other organic anions due to the paucity of relevant data needed for such corrections. Since this could significantly affect the calculated permeability ratios, none are presented. Substitution of fluoride for chloride irreversibly depolarized the preparation within 5 min in three experiments (cells 7, 8, 9 in Table I). Precipitation of calcium and magnesium in the barnacle saline by F^- was a possible but unlikely cause, since preparations studied in calcium and magnesium free barnacle saline were not irreversibly depolarized after more than 20 min in this solution. Further experiments are required on the F^- effect.

Iodide and bromide caused membrane hyperpolarization in preparations exposed to this series, with iodide producing the greatest change. I > Br > Cl in four preparations and no other sequence was observed with these halide anions. Isethionate appeared to be less permeant than chloride and more permeant than methanesulfonate in five preparations. Sulfate was found to be only somewhat less permeable than I^-. The least ambiguous sequence of these anions was:

$$I > SO_4 > Br > Cl > \text{isethionate} > \text{methanesulfonate}.$$

There was more variation in the ordering of gluconate, glucuronate, and glutamate with respect to chloride. Most often, propionate appeared less permeable than all of the anions tested but exceptions were observed as shown in Table I (cells 3 and 5). The best sequence for these anions appeared to be gluconate > glutamate > glucuronate > Cl. A summary of the complete sequence suggested by these experiments is presented at the bottom of Table I.

Paratoluene sulfonate (PTS) was tested as a potential anion substitute since it is less polar than its methane analog and might be expected to be less permeant. Partial substitution of PTS for chloride (i.e. replacing all NaCl with NaPTS) resulted in substantial (40 mV) but reversible depolarization that was associated with an increase in membrane conductance. Substitution of Tris PTS for Na PTS hyperpolarized the membrane 20 mV beyond the resting level in the NaCl saline. When a Na-free Tris Cl saline was replaced by a Na-free Tris PTS solution there was only a very slight membrane depolarization (+2 mV). This suggests that even though PTS is less permeable than Cl^-, the large depolarization observed in NaPTS is largely attributable to an increased Na conductance. If other anion substitutes exert a similar effect, calculated alkali-metal cation permeability ratios would be underestimated.

DISCUSSION

The assumption that intracellular cation activities remain constant during cation substitution experiments represents a potential problem for studies of the type described herein. Brown (1976) has reported that internal K^+ activity (a_K^i) increased quite readily in response to changes in $[K]_0$. In another study (Brown and Ottoson, 1976) it was reported that a_{Na}^i increases in response to K-free solutions. These changes require a longer exposure to the test cation solution than the exposure used in the present investigation. An increase in a_K^i as extracellular sodium is replaced by potassium would decrease the membrane potential change and the calculated K^+-Na^+ permeability ratios.

Changes in the junction potential of the extracellular reference electrode should introduce only small changes in the estimates of permeability ratios. The calculated junction potential changes would cause less than 4% underestimation of P_K/P_{Na} and P_{Li}/P_{Na}. The use of concentrations rather than activities introduces little error in comparison to the errors introduced by the other assumptions inherent in this treatment. Until internal ion activities are monitored under the present experimental conditions, the quantitative estimates of the permeability ratios should be considered minimal values; however, the cation permeability sequence established in this investigation should not be affected by the considerations mentioned above.

The permeability and conductance sequences appeared to be the same in the dark-adapted photoreceptor. This same sequence (Eisenman sequence IV) has been repeatedly observed in other classes of nerve cells (e.g., squid axon: Baker et al., 1962; muscle fibers: Hagiwara et al., 1971; for sequences observed for other biological and nonbiological phenomena: cf. Diamond and Wright, 1969). There was some difficulty in obtaining exact conductance ratios to compare with the permeability ratios, since the relation between conductance and concentration appears sigmoid and ions with low conductance (Cs^+ and Li^+) cannot be applied in sufficient concentration to evaluate the range of linearity and saturation. Membrane conductance in both K^+ and Rb^+ salines evidenced some saturation at concentrations greater than 200–300 mM. Cs^+ and Li^+ conductances showed little dependence on concentration over this range but increased (decreased) for a full substitution. For these reasons, the ratios of the relative conductance were obtained for a full substitution of the test cations where there was a clear departure from the conductance in the control solution. This produced conductance ratios that were in approximate agreement with the permeability ratios with the exception of K^+ which consistently had a higher conductance ratio. This could represent some voltage-dependent conductance change associated with the high level of depolarization in the K^+ solution, or the permeability ratios may be underestimated due to the considerations mentioned above.

The fact that the conductance and permeability ratios are nearly the same suggests that ion selectivity in the resting state is dominated by equilibrium factors and that the mobility ratios for the ions are close to unity (Eisenman, 1967). This is consistent with a neutral mechanism as described in the Introduction. However, the sigmoid relation between ion concentration and membrane conductance suggested by Fig. 4 is reminiscent of the ion-exchanger behavior described by Conti and Eisenman (1965). Further studies are being conducted that could discriminate between these alternatives.

The present literature indicates that the light-sensitive selectivity sequence is different from the resting state in *Balanus* photoreceptor. The light-induced current is carried largely, but not exclusively, by Na^+ ions (Brown et al., 1970); Li^+ can substitute to some extent for Na^+ (Koike et al., 1970) and K^+ was not observed to carry any significant portion of the light-induced current (Brown et al., 1970). Thus, a partial permeability sequence would be $P_{Na} > P_{Li} > P_K$. This also appears to be the sequence of *Limulus* ventral photoreceptors, on the basis of presently available data (Brown and Mote, 1974). If this is one of the well-known Eisenman sequences it can only be sequence X, while sequence IV was obtained for the resting state. Baker et al. (1962) found sequence IV for the resting state squid axon and Chandler and Meves (1965) found that the active currents in squid axon were sequence XI. Further studies should help to establish other differences between electrically excitable membranes and membranes that respond with graded potential changes.

Low pH increases anion conductance in *Balanus nubilus* muscle fibers (Hagiwara et al., 1968). The anion permeability and conductance sequences in these muscle fibers at pH 3.9 were found to be approximately the reverse of one another (Hagiwara et al., 1971). Anion permeability and conductance sequences

are essentially identical in frog skeletal muscle at pH 7.7 (Woodbury and Miles, 1973) and chloride conductance is increased at high pH (Hutter and Warner, 1967). Current-voltage relations during anion substitution (pH 7.6) in the present study showed no definitive conductance sequence. The anion permeability sequences of *Balanus* muscle fiber and photoreceptor membrane appear to be the same. This sequence ($I > Br > Cl$) corresponds to Eisenman's anion sequence I and is the order of free solution mobilities. This sequence has been associated with weak or widely spaced membrane sites (Diamond and Wright, 1969). It had been observed earlier (Brown et al., 1970) that the *Balanus* photoreceptor membrane was relatively insensitive to changes in extracellular chloride as Cl_0 was replaced by methanesulfonate. In the present study the same is true for a wide variety of anion substitutes. We have found that chloride washes out of the cell quite rapidly under conditions of Cl^- replacement, and that less permeant anions, such as propionate and methanesulfonate, enter the cell at the same time (Saunders and Brown, 1977). The membrane appears to be quite permeable to many anions yet the membrane has little electrical expression to anion changes. This suggests that an electrically silent mechanism such as exchange diffusion plays a role in Cl^- transport in *Balanus* photoreceptor.

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