Intracellular Na^+, K^+, and Cl^- Activities in *Balanus* Photoreceptors

H. MACK BROWN

From the Department of Physiology, University of Utah Medical Center, Salt Lake City, Utah 84132

ABSTRACT Ion-sensitive microelectrodes were used to measure intracellular activities (a^i_x) of Na^+, K^+, and Cl^- in *Balanus* photoreceptors. Average values of a^i_{Na}, a^i_K, and a^i_{Cl} were 28 mM, 120 mM, and 65 mM, respectively. Equilibrium potentials calculated from these average values were: Na^+ +64 mV, K^+ −77 mV, and Cl^- −42 mV; the average value of the resting potential for all cells examined was −41 mV. Long exposure to intense illumination produced measurable increases in a^i_{Na}. Classical $Na^+ - K^+$ reciprocal dilution experiments were analyzed with and without observed changes in a^i_K. As a^o_K was increased, the membrane depolarized, and a^i_K increased. Better agreement was found between the membrane potential and the directly determined E_K than expected from the standard relation between E_m and a^o_K. The latter produced pNa:pK estimates of the resting photoreceptor membrane that were higher than estimates based on data from the ion electrodes. Generally, E_m was more negative than E_K as a^o_K was increased. This is consistent with a significant chloride permeability in the dark-adapted photoreceptor.

INTRODUCTION

Ion-sensitive microelectrodes have permitted the direct measurement of intracellular, monovalent ion activity in axons (Hinke, 1961; Strickholm and Wallin, 1965), muscle cells (Hinke, 1959; Lev, 1964; Sorokina, 1964; Khuri, Hajjar, and Agulian, 1972), and large ganglion cells (Kostyuk, Sorokina, and Kholodova, 1969; Thomas, 1969, 1972; A. M. Brown. Walker, and Sutton, 1970; Kunze, Walker and Brown, 1971). Information concerning the intracellular ion activities of any type of receptor cell is scarce. Most receptor cells are relatively small and this is probably the main reason that internal ionic activities have not been investigated with ion-sensitive electrodes since the technique generally requires an independent measurement of the membrane potential with a second microelectrode. Intracellular ion concentrations have been estimated in some photoreceptors with whole tissue analysis (for example, see Hagins, 1972). These estimates are subject to some ambiguity because of the inherent difficulties of estimating intracellular water; in addition the tissue analysis was usually not confined to a homogeneous population of receptor cells. These data can only provide information concerning intracellular concentrations of important intracellular ions, whereas it would be desirable to know the activities of the ion species in question.

For the above reasons the present study was conducted to obtain values for the intracellular activities of Na^+, K^+, and Cl^- in single *Balanus* photoreceptors, to relate these measurements to similar measurements obtained in other types of cells and to the electrical behavior of this cell obtained by other electrophysiological techniques (Brown, Meech, Koike, and Hagiwara, 1969; Brown, Hagiwara, Koike, and Meech, 1970; Koike, Brown, and Hagiwara, 1971). Ion-sensitive microelectrodes have been used with success in this preparation in conjunction with the investigation of some electrical properties of this photoreceptor (Brown and Cornwall, 1975; Brown and Ottoson, 1976). The present study represents the first attempt to characterize "best values" for the intracellular ion activities of Na^+, K^+, and Cl^- in this receptor by obtaining relatively rapid measurements on a number of different cells so that extraneous factors would influence the results as little as possible.

In addition, direct measurements of a^i_K were obtained as a^o_K was varied. An analysis of these data forced the conclusion that pNa:pK obtained by more conventional methods would overestimate Na^+ permeability in the resting state in this preparation. These data were consistent with a significant Cl^- permeability that would not have been revealed without the aid of ion-sensitive electrodes.

METHODS

Preparation

Barnacle (*Balanus eburneus*) photoreceptors were prepared for electrophysiological recording as described previously (H. M. Brown et al., 1970). They were bathed in an artificial saline consisting of (mM): NaCl, 462; KCl, 8; $MgCl_2$, 12; $CaCl_2$, 20; Tris/Tris HCl, 10 (pH 7.62). The K^+ concentration was increased by mixing appropriate amounts of K^+-free saline containing 470 mM NaCl with a Na^+-free saline containing 470 mM KCl.

Electrodes

MEMBRANE POTENTIAL RECORDINGS A single photoreceptor was penetrated under visual control with two microelectrodes. One was used to record the membrane potential and the second to monitor the internal ion activity (a^i_x) where x = Na^+, K^+, or Cl^-. The former was usually filled with 3 M KCl. Several control experiments were conducted with other electrodes to determine whether or not any leakage from the KCl electrode would influence the measurements of a^i_x. a^i_K was determined in five cells with microelectrodes filled with 3 M NaCl and a^i_{Cl} was determined in another group of four cells with 4 M potassium acetate electrodes. Assurance that these electrodes provided an accurate measure of the membrane potential was obtained by penetration of a single cell with a KCl micropipette and an electrode filled with one of the other electrolytes. The membrane potential obtained with the potassium acetate and NaCl electrodes was within ± 1 mV of that recorded with the KCl electrode.

Two methods were used to ensure that both the ion-sensitive electrode and membrane potential electrode were in the same cell. In the first instance, small (10 namp) inward-going current pulses were temporarily passed across the membrane by using the bridge circuit shown schematically in the inset of Fig. 1. The second method involved illumination of the cell and noting the potential change recorded from electrodes after the decline from the peak of the light response. If any differences were noted in the potential changes recorded from the E_m electrode or the ion-sensitive electrodes under either of these conditions one or the other electrode was moved to a new location until the criteria

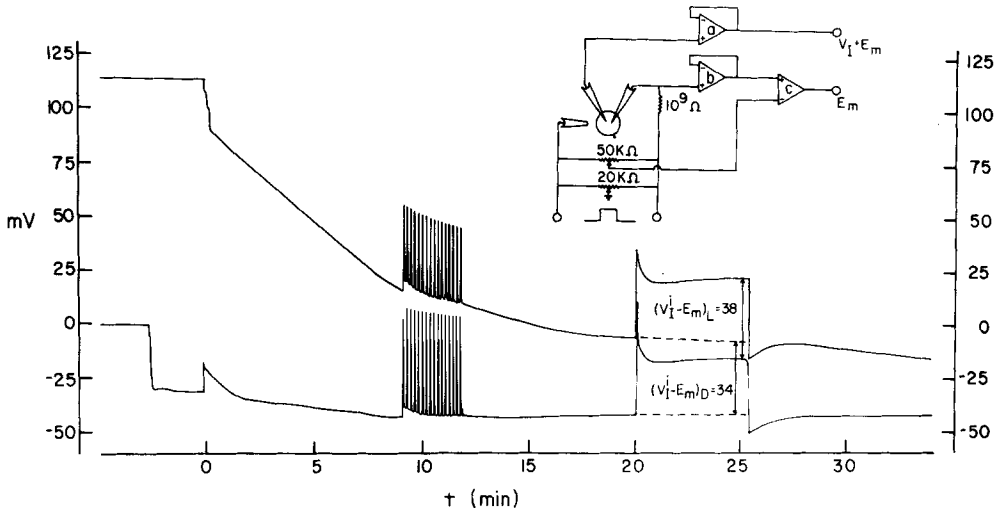

FIGURE 1. The record shows membrane potential changes (bottom trace) during penetration of the cell with a KCl microelectrode and 3 min later (0 time) the potential changes (upper trace) of a Na^+-sensitive microelectrode following penetration of the cell. Brief light flashes were presented about 9 min after penetration with both electrodes: a long light flash was presented 20 min after penetration. The decrease in the difference between the recorded voltages of the ion electrode and the membrane potential electrode ($V_I^i - E_m$) after penetration reflects a decrease in a^i_{Na}. This difference became greater during illumination which indicates a^i_{Na} increased (see text).

Inset: Recording arrangement to ascertain that potential changes recorded from the membrane potential (E_m) recording electrode and the ion-sensitive electrode (V_I^i) were the same when current pulses were passed across the membrane. *a*, unity gain high impedance (10^{14} ohms) varactor bridge amplifier; *b*, unity gain capacity neutralized preamplifier (10^{11} ohms); *c*, differential amplifier for cancellation of potential drop across recording microelectrode during current pulses.

were met. Another limit set on acceptance of data from this study was agreement of the pre- and postcalibration curves of the ion-sensitive electrodes. Approximately 40% of the experiments attempted met all these qualifications and are reported here.

ION-SENSITIVE MICROELECTRODES Liquid ion-exchanger microelectrodes were constructed to measure K^+ and Cl^- activities (Corning exchanger 477317 and 477315, respectively) as described by Orme (1969) and Walker (1971). The selectivity of these electrodes for several competing ions of possible significance has been reported (Orme, 1969; Walker, 1971). Selectivity coefficients ($k_{K,Na}$) for the K^+ electrodes used in the present study were determined routinely from reciprocal dilution of Na^+ and K^+ (as chlorides at 0.1 M) and the values were generally about 0.02. The Na^+ error in most a^i_K determinations should be less than 1% with this selectivity.

The measured Cl^- activities were not corrected for interference since the kind and amount of interfering anions are unknown in these cells. However, measurements of a^i_{Cl} with these electrodes may be more uncertain than those obtained with the K^+ and Na^+ electrodes since they have appreciable sensitivity to certain other anions of biological significance (Walker, 1971; Owen, Brown, and Saunders, 1975; Saunders and Brown,

1975). For example $k_{Cl,isethionate}$ was about 0.20 and k_{Cl,HCO_3} was usually between 0.10 and 0.15 for electrodes used in this study.

Na^+-sensitive microelectrodes were constructed from Corning NAS 11-18 glass (Eisenman, 1965) according to the methods of Hinke (1959) and Thomas (1970, 1972). The most successful of the two types was the recessed tip electrode since the tip diameters (approximately 1.0 μ) were smaller than the drop-through type and thus they more easily penetrated the cell. The micro-well in the tip of the recessed tip electrode was less than 1% of the volume of a 100 μ sphere. The selectivity ($k_{Na,K}$) of these electrodes for Na^+ over K^+ was determined routinely from reciprocal dilution of K^+ and Na^+ (as chlorides) at constant ionic strength (0.1 M). Values of $k_{Na,K}$ for different electrodes ranged between from 5×10^{-3} to 1×10^{-3}. A small error was possible in calculating a^i_{Na} for certain of the Na^+ electrodes, since intracellular a^i_K is relatively high compared to the level of intracellular Na^+. Therefore, the a^i_{Na} measurements were corrected routinely by an appropriate amount depending upon the $k_{Na,K}$ obtained for a particular Na^+ electrode.

DETERMINATION OF a^i_x. The membrane potential and ion-sensitive electrodes were led off via Ag/AgCl half-cells to unity gain probes with suitable input impedance as shown in the inset of Fig. 1. The input impedance of amplifier a and b was 10^{14} and 10^{11} ohms, respectively. The differential amplifier c was required to cancel the potential drop across the recording microelectrode when current pulses were applied. The membrane potential was displayed on a CRO and one channel of a chart recorder; the ion probe was led off to a digital voltmeter and a second channel of the chart recorder. The chart record provided a convenient means of visualizing changes in ion activity when the membrane potential was set to zero reference potential while outside the cell and the ion electrode assumed some value (V^o_i) with respect to this reference in the external saline. When both electrodes were inside the cell the difference in potential between the two electrodes ($V^i_i - E_m$) was used to calculate the internal ion activity (a^i_x; x = Na^+, K^+, or Cl^-) from the relation:

$$a^i_x = a^o_x \cdot \exp \frac{(V^i_i - E_m) - V^o_i}{m} - k_{xy} a^i_y \qquad (1)$$

where V^o_i is the potential of the ion electrode in the external saline with primary ion activity a^o_x; V^i_i is the potential of the ion electrode inside the cell; m is dV/da_x of the ion-sensitive electrode after calibration in pure solutions of the primary ion x; k_{xy} is the selectivity of the electrode for the competing ion, y, with respect to the primary ion, x, and a^i_y is the activity of the competing ion.

RESULTS

Internal Na^+ Activity

Fig. 1 shows a record of the potential changes recorded upon penetration of the membrane with a standard KCl microelectrode (bottom trace) and a Na^+-sensitive microelectrode (top trace). First the cell was penetrated with the KCl electrode and 3 min later it was penetrated with the Na^+-sensitive microelectrode. Time zero corresponds to the time that both electrodes were in the cell. Upon penetration of the cell with the Na^+-sensitive microelectrode the membrane depolarized, but recovered within about 2 min. The initial potential change of the Na^+ electrode was fairly abrupt and was followed by a much slower decrease of the electrode potential. The former probably represents the sensing of the membrane potential by the ion electrode upon penetration of the cell and the latter phase the loss of Na^+ from the micro-well in the tip of the electrode to

the cell interior. The reference potential for the KCl microelectrode was zero while located in the external bath whereas the Na^+ electrode yielded a large positive value with respect to this reference value which corresponded to the high level of Na^+ in the external saline (a^o_{Na} = 315 mM). Penetration of the cell with the Na^+ electrode produced a reduction in the "distance" between the two traces in Fig. 1 and this distance corresponds to the $(V_l^i - E_m)$ values used to calculate a^i_{Na} from eq. 1. Potential changes to 1 s flashes of light at 10 s intervals are shown several minutes after the cell was penetrated with both electrodes. The peak transient phase of the potential change to light was attenuated in the recording from the Na^+ electrode due to the low frequency response of the probe used for that electrode; this presented no problem in the present study since steady-state potential values were the only ones of interest. It is worth noting that there was a slight elevation of the Na^+ potential following these few light flashes, whereas the membrane potential resumed its earlier value after the light flashes. The slight increase in $(V_l^i - E_m)$ reflects a small increase in a^i_{Na}. This is more easily seen when the Na^+ electrode had attained a more steady value (20 m after penetration) and a long light flash was used. The interrupted lines represent the recorded voltage from the Na^+ electrode and the membrane potential electrode had the cell been kept in darkness. The value of $(V_l^i - E_m)_D$ was 34 mV which yields an a^i_{Na} of 13 mM (eq. 1). However, the potential difference recorded after 6 m of illumination $(V_l^i - E_m)_L$ was 38 mV which yields a value of 15.4 mM for a^i_{Na}; thus, there was a net increase in a^i_{Na} of 2–3 mM during the exposure to light in this cell. A similar exposure to light in some other cells increased a^i_{Na} by as much as 5 mM.

Final values of a^i_{Na} in the resting state were obtained when there was no change in the resting membrane potential and the reading of the ion-sensitive microelectrode. This usually varied from 30 min to an hour for the Na^+ electrode, and from 10 to 20 min for the K^+ and Cl^- liquid ion-exchanger electrodes. The measured a^i_{Na} in Fig. 1 declined with a time constant of about 9 min following penetration of the cell with the Na^+ electrode.

A likely reason that the Na^+ electrodes required a longer time to reach steady values than the exchanger electrodes, was that a high concentration of Na^+ was carried from the external medium into the cell in the "well" of the electrode and loss of Na^+ from the well may have been retarded due to some plugging of the electrode tip during penetration. It was often observed upon removal of the Na^+ electrode from the cell that the electrode required much longer (5–10 min) to reach a steady-state in the calibrating solutions than it did prior to insertion in the cell (1–2 min). Washing the electrode often rectified this condition. Reduction of a^i_{Na} by cellular pumping mechanisms could also be involved but the time constants of a^i_{Na} after penetration with Na^+ electrodes were generally at least twice as long as those observed for changes in a^i_{Na} due to a presumptive electrogenic pump in this receptor (Koike et al., 1971; Brown and Ottoson, 1976).

Table I shows values of internal Na^+ activity (a^i_{Na}) measured in 13 different cells when steady-state values of the membrane potential and the potential of the ion electrode were attained. The time required to reach the final steady value in darkness is indicated in the fifth column (t). There was no apparent correlation

TABLE I

INTERNAL SODIUM ACTIVITY (a^i_{Na}) MEASURED IN 13 DIFFERENT CELLS WITH 13 DIFFERENT Na^+-SENSITIVE ELECTRODES

Cell	a^i_{Na}	E_{Na}	E_m	t
		mV	*mV*	*min*
1	8	+92	−41	55
2	11	+85	−41	20
3	12	+82	−35	90
4	23	+66	−42	48
5	23	+66	−41	32
6	23	+66	−42	30
7	28	+61	−42	30
8	28	+61	−36	49
9	28	+61	−47	30
10	40	+52	−37	30
11	40	+52	−43	36
12	49	+47	−41	54
13	50	+46	−35	40
$\bar{y}$	28	+64	−40	42
SD (±)	13.6	14.4	3.5	18

Data are ranked from the lowest to highest value of $a^i_{Na} \cdot E_{Na}$ (column 3) calculated from $E_{Na} = 58 \log_{10} (315 \text{ mM}/a^i_{Na})$. The membrane potential measured with a KCl electrode is shown in column 4. The time required to attain a steady-state a^i_{Na} after penetration of the cell is indicated in column 5.

between (t) and the final value of a^i_{Na}. For example, similar values of a^i_{Na} were obtained from cells 2 and 3 (11 and 12 mM, respectively) but the former required 20 min and the latter 90 min to reach the steady-state value. The range of a^i_{Na} in darkness was 8 to 50 mM, yielding calculated values of E_{Na} from +92 to +46 mV with a mean value of +64 mV ± 13.6 mV (SD). The resting potential (E_m) of these cells compares favorably with that obtained previously during impalement with two smaller KCl-filled pipettes (H. M. Brown et al., 1970) and the smaller ion-exchanger microelectrodes used in this study.

The electrodes were withdrawn from the cell immediately after the steady-state values had been obtained to reduce the possibility of changes in the reference potential measured outside the cell. The ion-sensitive electrode was recalibrated immediately after a steady-state value in the external saline was attained.

Internal Chloride Activity

Measurements of a^i_{Cl} were obtained by the same experimental procedure outlined in Fig. 1 but with Cl^--sensitive ion-exchanger microelectrodes. Table II presents a summary of the results obtained from nine cells and nine different Cl^--sensitive electrodes. A wide range of a^i_{Cl} values was obtained from different cells as seen in column 2 (94 to 43 mM). However, by comparing columns 3 and 4 it can be seen that E_{Cl} and the resting potential (E_m) for a given photoreceptor were close to the same value. This is evidence that Cl^- ions are distributed passively in the dark-adapted barnacle photoreceptor or alternatively that Cl^-

permeability is dominant in the resting photoreceptor. There was no statistically significant difference between the mean values of E_{Cl} and E_m. The cell by cell differences between E_{Cl} and E_m are shown in column 5 and are considered to be within the range of uncertainty of the measurement technique. The largest difference (cell 1) would represent a difference of only 4 mV between V_I^i and E_m. There was little evidence of any change in a^i_{Cl} when the cell was illuminated for the same period of time required to elicit a change in a^i_{Na} (about 5 min). Therefore, even though Cl^- seems to be at equilibrium in the dark-adapted state, there is no immediate change in a^i_{Cl} when the membrane potential is altered with illumination.

Internal K^+ Activity

Steady-state values for a^i_K obtained with 11 separate K^+-sensitive liquid ion-exchanger electrodes in 11 cells are shown in Table III, column 2. As with the Cl^- and Na^+ measurements, the intracellular values of a^i_K were obtained as soon as steady values were reached so that a^i_K could be established and the electrodes recalibrated within a short time. Usually this required no more than 60 min after the initial penetration of the cell with both microelectrodes.

A rather wide range of a^i_K was obtained from different cells: cell 1 had a value of only 86 mM whereas cell 11 had a value of 138 mM. However, as seen from column 3 the calculated values of E_K were only 12 mV different between each extreme. The average values of a^i_K and E_K shown at the bottom of the table were 120 mM and −77 mV, respectively. Unlike E_{Cl} E_K was consistently more negative than E_m: the difference between E_K and E_m for individual cells is shown in column 5 and the difference of the mean values was −35 mV. The range of values for the resting membrane potential (E_m) in these cells is comparable to

TABLE II

INTERNAL CHLORIDE ACTIVITY (a^i_{Cl}) FROM NINE CELLS AND NINE DIFFERENT Cl^--SENSITIVE ELECTRODES

Cell	a^i_{Cl}	E_{Cl}	E_m	$E_{Cl} - E_m$
	mM	*mV*	*mV*	
1	94	−32	−28	−4
2	76	−38	−40	+2
3	72	−39	−40	+1
4	60	−43	−40	−3
5	68	−40	−41	+1
6	67	−41	−41	0
7	59	−44	−43	−1
8	49	−48	−44	−4
9	43	−52	−51	−1
$\bar{y}$	65	−42	−41	−0.1
SD (±)	15.1	5.8	6.0	2.5

Data are ranked according to the magnitude of the steady-state resting potential in darkness (column 4). Column 3 (E_{Cl}) calculated from $E_{Cl} = 58 \log_{10} (a^i_{Cl}/325$ mM); 325 mM was the average value of barnacle saline from several independent determinations with Ag/AgCl electrodes. The difference between the mean values of E_{Cl} and E_m shown in column 5 was not significant (t-test).

TABLE III

INTERNAL POTASSIUM ACTIVITY (a^i_K) MEASUREMENTS FROM 11 CELLS WITH 11 DIFFERENT K^+-SENSITIVE ELECTRODES

Cell	a^i_K	E_K	E_m	$E_K - E_m$
	mM	*mV*	*mV*	
1	86	−69	−39	−30
2	98	−71	−45	−26
3	109	−75	−39	−36
4	109	−75	−44	−31
5	114	−76	−41	−35
6	130	−80	−44	−36
7*	130	−80	−38	−42
8	132	−80	−46	−34
9	134	−80	−44	−36
10	135	−81	−46	−35
11	138	−81	−40	−41
$\bar{y}$	120	−77	−42	−35
SD (±)	17.3	4.2	3.0	4.6

Data are ranked from lowest to highest values of a^i_K. The values of E_K (third column) calculated from $E_K = 58 \log_{10} (5.5 \text{ mM}/a^i_K)$. The membrane potential (E_m) at the time the a^i_K was obtained is shown in the fourth column. Differences between E_K and E_m are indicated in column 5.

* Double barrel (Khuri, Hajjar, and Agulian, 1972).

that in which a^i_{Na} and a^i_{Cl} were measured. There appears to be little correlation between the calculated E_K and the resting potential. The rather large difference between E_K and E_m at the resting state in darkness indicates that the membrane is significantly permeable to ions other than K^+. For that matter it could be tentatively argued from a comparison of E_{Cl} and E_m that Cl^- is the most permeable ion species. A discussion of the role that other ions play in the maintenance of the resting potential is presented in the next section.

a^i_K and Relative Permeability of Na^+, K^+, and Cl^-

It is commonly observed that as the external K^+ concentration is raised the potential change of the membrane is less than expected from the behavior of an ideal K^+ electrode: the dark-adapted barnacle photoreceptor is no exception (H. M. Brown et al., 1970). This deviation is usually attributed to a significant permeability of the resting membrane to Na^+. The classical approach in the estimation of the relative contribution of Na^+ and K^+ to the resting potential is to rearrange the Goldman, Hodgkin, Katz equation so that a Na:K permeability ratio (α) can be calculated:

$$\exp \frac{E_m F}{RT} = \frac{\alpha \cdot M}{a^i_K} + \frac{1 - \alpha}{a^i_K} \cdot a^o_K \qquad (2)$$

where $M = a^o_{Na} + a^o_K$ and F, R, and T have their usual meanings. This calculation of α rests on the assumptions that (1) a^i_K is relatively constant at all concentrations of external potassium, (2) that the value of a^i_K can be specified

from the relation between E_m and a^o_K, and (3) that the Cl^- permeability is insignificant.

The use of the K^+-sensitive microelectrode in conjunction with ion substitution experiments provides a convenient method of evaluating the validity of these assumptions directly. This was done on the barnacle photoreceptor and the first order approximation of α from the E_m vs. a^o_K relation was found to require significant modification since evidence was obtained that all three assumptions are questionable under the present experimental conditions.

Fig. 2 shows changes of the membrane potential (E_m) as the external NaCl was replaced with KCl (M = NaCl + KCl = 320 mM). After each exposure of the cell to a higher a^o_K the cell was returned to normal saline as indicated by multiple data points at a^o_K = 5.5 mM. As the a^o_K was increased the membrane was depolarized; the maximum rate of membrane potential change per decade a^o_K was 43 mV. The range in different cells studied in this manner was from 42 to 48 mV.

From Fig. 2 and equation 1, a pNa:pK value of 0.13 was obtained for this cell. This should be considered a first approximation at best since data obtained from measurements of a^i_K with K^+-sensitive electrodes under the same experimental conditions revealed that rather large changes in a^i_K occur under these conditions. This is shown in Fig. 3 A for two different photoreceptors; solid circles correspond to data from the same cell shown in Fig. 2. As a^o_K was increased the a^i_K also increased significantly. The same was true for the other cell which had a higher initial a^i_K in normal saline (solid squares). The increase was especially apparent for changes in a^o_K from 5.5 mM to 160 mM; at higher values of a^o_K, a^i_K remained relatively constant. Thus, as a^o_K was increased to 160 mM, the value of a^i_K approached the same value. From these data, assumption (1) that a^i_K remains constant as a^o_K is increased was clearly violated. From a comparison of data in Figs. 2 and 3 A, assumption (2) also appears to be violated. That is, the value of a^i_K obtained from Fig. 2 overestimates the true a^i_K. The intersection of the curve

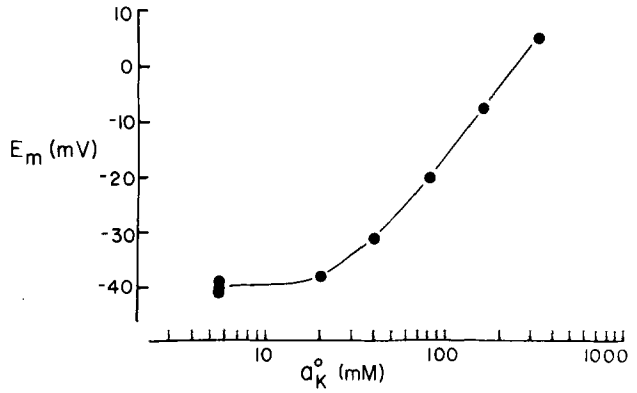

FIGURE 2. Membrane potential of barnacle photoreceptor (vertical axis) as a^o_K was increased (horizontal axis). The preparation was returned to normal saline (a^o_K = 5.5 mM) after each increment in a^o_K. The rate of membrane potential change was 43 mV/decade a^o_K at the highest activities of a^o_K.

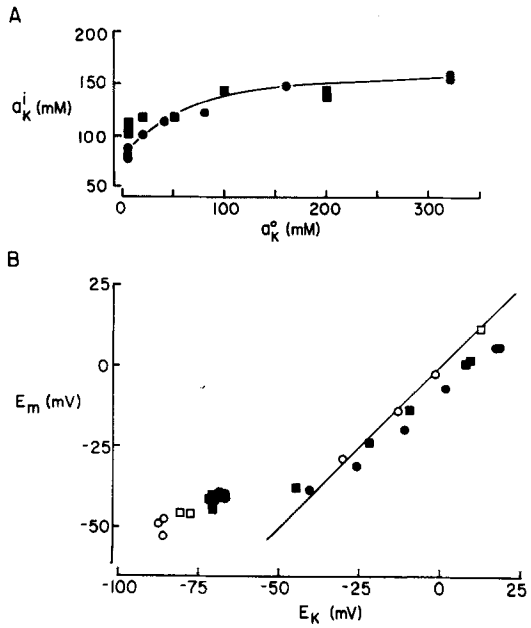

FIGURE 3. A, internal K^+ activity (a^i_K) measured with liquid ion-exchanger microelectrodes as the external K^+ activity (a^o_K) was raised in the external saline. Two cells; solid circles same cell as Fig. 2. B, relationship between membrane potential (E_m) and (E_K) calculated from measurements of internal and external K^+ activity with liquid ion-exchanger microelectrodes. Four cells; solid circles same cell as Fig. 2. The calculated values of E_K were obtained when E_m and a^i_K had attained steady-state values after a change in a^o_K.

in Fig. 2 with 0-membrane potential corresponds to an a^i_K of 245 mM. The value of a^i_K measured directly with the K^+ electrode in Fig. 3A was about 160 mM at the same a^o_K. Consequently the α obtained previously clearly requires revision since the values derived from data in Fig. 2 do not correspond to experimentally determined values. This is not unexpected if the relationship between E_m and experimental values of E_K at different a^o_K's is examined. This relationship is shown in Fig. 3 B for four different cells. Data from the cell shown in Figs. 2 and 3 A are represented by solid circles. The solid line represents a perfect correspondence between E_m and E_K. As the a^o_K was increased the correspondence between E_m and E_K was much better than would have been predicted from Fig. 2 or α calculated from eq. 1. The data in Fig. 3 B suggest that pNa is significantly lower than previously estimated, i.e., the membrane is a better K^+ electrode than expected; moreover, there is a unique membrane potential value (approx. −35 mV) where $E_m = E_K$. A revised estimate of pNa:pK (α') can be obtained from data based on directly measured values of a^i_K as a^o_K was varied:

$$\alpha' = \exp \frac{E_m F}{RT} \cdot \frac{(5 + a^i_K) - a^o_K}{a^o_{Na}} \tag{3}$$

where a^i_K and a^o_K were measured directly (Figs. 2 and 3 A) and $a^o_{Na} = 320 - a^o_K$; 5

is the product of a^i_{Na} and α. This product is an upper limit based on the highest value of a^i_{Na} obtained in this investigation (Table I) and the average α from a series of experiments using eq. 2. The estimate of pNa:pK calculated from (3) at the resting potential (E_m = −40 mV) is considerably less than that obtained previously from eq. 2 (0.04 vs. 0.13), but the ratio varies depending on a^o_K (and/or E_m). Table IV, column 3 shows α' calculated for four different membrane potential values. The negative values at E_m = −8 mV and 0 mV indicate that the emf of some ion species besides Na^+ and K^+ is significant. This is reflected in the relationship between E_m and E_K in Fig. 3 B, i.e., for the smaller increases of a^o_K the membrane potential was more positive than E_K($E_m > E_K$). This would yield positive α' values. But at greater a^o_K's the membrane potential was more negative than E_K($E_m < E_K$); i.e., the E_K values for all cells fell to the right of the unity slope line. This was most conspicuous in cells represented by the solid squares and circles and would correspond to negative α' values. This reversal of E_m with respect to E_K as a^o_K was increased can only be accounted for by a significant Cl^- permeability since the Cl^- equilibrium potential is the only one with the appropriate sign to yield E_m's more negative than E_K or E_{Na} (with the exception of those data obtained from full substitutions of K^+ for Na^+). This membrane potential behavior is explicable if pCl is considerably greater than pNa under these conditions; i.e., pK > pCl ≫ pNa. If experimental values of a^i_K at different a^o_K's and the experimentally determined mean value of a^i_{Na} (25 mM) are used in conjunction with pK = 1, pCl = 0.3, and pNa = 0.04, good agreement between the calculated and actual membrane potential is obtained for the present experiments as is shown in Table IV, column 4. The inclusion of a Cl^- permeability can account for the shift in the relative position of E_m with respect to E_K shown in Fig. 3 B. Chloride permeability cannot be considered to be inconsequential from the present experimental results and assumption (3) underlying the use of eq. 1 appears to be violated. Data obtained from the K^+-sensitive electrodes make the assumptions unnecessary since experimentally derived values can be used to provide an estimate of relative ion permeabilities.

TABLE IV

REVISED pNa:pK RATIOS (α' IN COLUMN 3) AT DIFFERENT MEMBRANE POTENTIALS (COLUMN 1) BASED ON VALUES OF a^i_K MEASURED WITH K^+-SENSITIVE MICROELECTRODES

E_m*	E_K	α'‡	E_m§
−40	−70	0.04	−40
−36	−36	0.0	−33
−8	0	−0.26	−8
0	+10	−0.72	+1

The experimental E_K's at each E_m are indicated in column 2. Column 4 shows calculated values of E_m based on permeability estimates obtained from experimental values of a^i_K where a Cl^- permeability term has been included.

* Fig. 2 solid data points.

‡ Eq. 3 in text.

§ Calculated from measured values of a^i_K, a^o_K, a^i_{Na}, a^i_{Cl}, and pK:pNa:pCl = 1:.04:0.3.

DISCUSSION

Results from the present experiments cast some doubt on the uncritical use of the GHK equation for estimation of permeability ratios in the barnacle photoreceptor. The finding that a^i_K increased significantly as a^o_K was increased is contrary to the major assumption underlying the use of the equation. If a^i_K is assumed to remain constant and specified from a plot like Fig. 1, the overall effect is that eq. 1 significantly overestimates Na permeability in the resting state. When experimental values of a^i_K and a^i_{Na} are used instead, the pNa:pK obtained is only about one-third of that obtained with eq. 1; i.e., the membrane was found to be a better K^+ electrode than expected from Fig. 1.

The second point of interest was that deviation of the membrane potential from E_K at the higher a^o_K's was not explicable exclusively on the basis of Na^+ and K^+ permeabilities whereas it was at lower a^o_K's; i.e., the membrane potential assumed values more positive than E_K at low a^o_K's and more negative values than E_K at the higher a^o_K's. This result was compatible with a significant Cl^- permeability since only E_{Cl} had an appropriate negative sign under these conditions. It was concluded previously (H. M. Brown et al., 1970) from alteration of $(K)_o$ at constant $(K)_o \times (Cl)_o$ that Cl^- permeability is less than K^+ permeability but relative values of the two were not obtained. From the present experiments Cl^- appears to be about one-third as permeable as K^+. A significant Cl^- permeability is contrary to another crucial assumption underlying use of eq. 1. It is difficult to say with any certainty from the present results whether or not Cl^- permeability can be considered to be negligibly low in the normal resting state and that as the membrane was depolarized in the high K^+ solutions the Cl^- permeability became more significant than the Na^+ permeability. Since the membrane potential behavior could be quite adequately calculated on the basis of fixed permeabilities to K^+, Cl^-, and Na^+ in the ratios 1:0.3:0.04 a permeability mechanism independent of membrane potential and a^o_K is tentatively suggested. A significant Cl^- permeability in the resting state could account for the observation (Tables I and III) that cells with low or high a^i_{Na} or a^i_K had resting potentials very close to −40 mV. If one attempts to reconcile these measurements solely on pNa:pK values the paradoxical conclusion is reached that photoreceptors which appear "leaky" or have an impaired Na^+-K^+ exchange pump evince better resting selectivity of K^+ over Na^+ ions. If it is assumed that a cell with the lowest measured a^i_{Na} has the highest a^i_K, then the calculated reciprocal α' (eq. 3) would be only 15:1, whereas a cell with the highest measured a^i_{Na} and lowest a^i_K would yield a value of 20:1.

Relevance of Internal Ion Activity to Membrane Mechanisms

It is well established in electrically excitable membrane with a sodium-dependent action potential that the active phase of membrane current reverses sign at a membrane potential very close to E_{Na} (Hinke, 1961) and that the action potential is abolished in Na-free solutions (Hodgkin and Huxley, 1952). Receptors in general also become more permeable to Na^+ during appropriate stimulation, but unlike squid axon some receptor activity can generally be elicited even in Na^+-free solutions (Kikuchi, Naito, and Tanaka, 1962; Edwards, Terzuolo, and Washizu, 1963; Ottoson, 1964; Millecchia and Mauro, 1969; H. M. Brown et al., 1970).

It has been possible to obtain measurements of light-induced membrane current over a wide range of membrane potentials in *Balanus* photoreceptors (H. M. Brown et al., 1970). The reversal potential becomes less positive as the external Na^+ concentration is reduced, but unlike the squid axon the barnacle membrane does not behave as an ideal Na^+ electrode. Values of E_{Na} obtained in the present study (Table I) are much more positive than the membrane potential at which light-induced current reverses sign in this receptor (average value of E_{Na}: +64 mV vs. +27 mV for the reversal of LIC). Thus, the barnacle photoreceptor appears to maintain a steep Na^+ gradient across the membrane similar to other nerve cells, but the disparity between the reversal potential of the active phase of membrane current and E_{Na} infers that another ion species besides Na^+ and Ca^+ (H. M. Brown et al., 1970) is involved in the light response (unless the Ca^{++} equilibrium potential is much less positive than anticipated).

The present measurements are also relevant to the post-illumination hyperpolarization (PIH) observed in this receptor. Under conditions resulting in an increase of a^i_{Na}, such as illumination (Fig. 1) or iontophoretic injection of sodium (Koike et al., 1971), the membrane potential of the receptor can become as much as 60–80 mV more negative than the resting potential prior to illumination (−40 to −50 mV). The equilibrium potentials obtained in the present investigation for Cl^- and K^+ (Tables II and III) were not sufficiently negative to account for this behavior on the basis of a conductance increase to either ion species. This strengthens the conclusion made earlier without specific knowledge of the ion distribution across the barnacle photoreceptor membrane, that the large hyperpolarization following a light flash is likely due to electrogenic pump activity (Koike et al., 1971).

Comparison with Ion Activities in Other Excitable Tissues

In normal saline, the dark-adapted photoreceptor membrane potential was always more positive than E_K which is in accord with findings in other classes of excitable cells including axon (Hinke, 1961), muscle (Kostyuk et al., 1969; Khuri et al., 1972), and large ganglion cells (Kostyuk et al., 1969; Brown et al., 1970; Kunze et al., 1971).

Chloride appears to be distributed passively in dark-adapted photoreceptors; i.e., $E_{Cl} = E_m$. *Balanus* photoreceptors share this property with skeletal muscle fibers (Boyle and Conway, 1941; Hodgkin and Horowicz, 1959). In axons, E_{Cl} has been found to be more positive than E_m (Strickholm and Wallin, 1965), whereas E_{Cl} of molluscan neurons has been reported to be more positive or more negative than E_m depending on the functional type of cell studied (Kerkut and Meech, 1966). However, even this is in question at present since recent experiments on snail neurons (Neild and Thomas, 1974) suggest that E_{Cl} is more negative than E_m despite the type of cell.

The lower values of sodium activity found in *Balanus* photoreceptors are comparable to those found in axon (Hinke, 1961), muscle (Hinke and McLaughlin, 1967), and ganglion cells (Brown and Brown, 1972) of other seawater species. The mean value of E_{Na} is of the same magnitude as that in nerve cells with quite a different value of a^o_{Na}, e.g. frog muscle (Sorokina, 1964; Kostyuk et

al., 1969) and snail neurons (Sorokina, 1964; Kostyuk et al., 1969; Thomas, 1969, 1972).

CONCLUSION

These results indicate that Na^+ and K^+ are distributed across this receptor membrane as in most other classes of excitable cells; Cl^- appears to be distributed passively. Under conditions of reciprocal changes of Na^+ and K^+, potassium ions enter the cell. The calculations illustrate some difficulties associated with estimating the magnitude of relative permeabilities in this receptor from classical ion substitution studies. Measurements of K^+ activity under these experimental conditions allow a more accurate estimate of pNa:pK values and indirectly indicated that under the present experimental conditions there is a significant Cl^- permeability in the resting state.

The careful technical assistance of Toni Gillett is greatly appreciated. The construction of the Na^+ electrodes was demonstrated by R. C. Thomas.
This work was supported by a grant from the National Eye Institute, EY 00762.

Received for publication 1 December 1975.

REFERENCES

BOYLE, P. J., and E. J. CONWAY. 1941. Potassium accumulation in muscle and associated changes. *J. Physiol. (Lond.)*. **100:**1–63.

BROWN, A. M., J. L. WALKER, and R. B. SUTTON. 1970. Increased chloride conductance as the proximate cause of hydrogen ion concentration effects in *Aplysia* neurons. *J. Gen. Physiol.*, **56:**559–582.

BROWN, H. M., and A. M. BROWN. 1972. Ionic basis of the photoresponse of *Aplysia* giant neuron: K^+ permeability increase. *Science (Washington, D. C.)*. **178:**755–756.

BROWN, H. M., and M. C. CORNWALL. 1975. Ionic mechanism of a quasi-stable depolarization in barnacle photoreceptor following red light. *J. Physiol. (Lond.)*. **248:**579–593.

BROWN, H. M., S. HAGIWARA, H. KOIKE, and R. W. MEECH. 1970. Membrane properties of a barnacle photoreceptor examined by the voltage clamp technique. *J. Physiol. (Lond.)*. **208:**385–413.

BROWN, H. M., R. W. MEECH, H. KOIKE, and S. HAGIWARA. 1969. Current-voltage relations during illumination: photoreceptor membrane of a barnacle. *Science (Washington, D. C.)*. **166:**240–243.

BROWN, H. M., and D. OTTOSON. 1976. Dual role for K^+ in *Balanus* photoreceptor: antagonist of Ca^{++} and suppression of light induced current. *J. Physiol. (Lond.)*. **257:** 355–378.

EDWARDS, C. C., A. TERZUOLO, and Y. WASHIZU. 1963. The effects of changes of the ionic environment upon an isolated crustacean sensory neuron. *J. Neurophysiol.* **26:**948–957.

EISENMAN, G. 1965. The electrochemistry of cation sensitive glass electrodes. *In* The Glass Electrode. G. Eisenman, R. Bates, G. Mattock and S. M. Friedman, editors. Interscience Publishers Inc., New York.

HAGINS, W. A. 1972. The visual process: excitatory mechanisms in the primary receptor cells. *Annu. Rev. Biophys. Bioeng.* **1:**131–158.

HINKE, J. A. M. 1959. Glass microelectrodes for measuring intracellular activities of sodium and potassium. *Nature (Lond.)*. **184:**1257–1258.

HINKE, J. A. M. 1961. The measurement of sodium and potassium activities in the squid axon by means of cation-selective glass microelectrodes. *J. Physiol. (Lond.)*. **156:**314–335.

HINKE, J. A. M., and S. G. A. MCLAUGHLIN. 1967. Release of bound sodium in single muscle fibers. *Can. J. Physiol. Pharmacol.* **45:**655–667.

HODGKIN, A. L., and P. HOROWICZ. 1959. The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *J. Physiol. (Lond.)*. **148:**127–160.

HODGKIN, A. L., and A. F. HUXLEY. 1952. Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo*. *J. Physiol. (Lond.)*. **116:**449–472.

KERKUT, G. A., and R. W. MEECH. 1966. The internal chloride concentration of H and D cells in the snail brain. *Comp. Biochem. Physiol.* **19:**819–832.

KHURI, R. N., J. J. HAJJAR, and S. K. AGULIAN. 1972. Measurement of intracellular potassium with liquid ion-exchange microelectrodes. *J. Appl. Physiol.* **32:**419–422.

KIKUCHI, R., K. NAITO, and I. TANAKA. 1962. Effect of sodium and potassium ions on the electrical activity of single cells in the lateral eye of the horseshoe crab. *J. Physiol. (Lond.)*. **161:**319–343.

KOIKE, H., H. M. BROWN, and S. HAGIWARA. 1971. Hyperpolarization of a barnacle photoreceptor membrane following illumination. *J. Gen. Physiol.* **57:**723–737.

KOSTYUK, P. G., Z. A. SOROKINA, and Y. D. KHOLODOVA. 1969. Measurement of activity of hydrogen, potassium and sodium ions in striated muscle fibers and nerve cells. *In* Glass Microelectrodes. M. Lavallee, O. Schanne, and N. C. Hebert, editors. John Wiley & Sons, New York.

KUNZE, D. L., J. L. WALKER, and H. M. BROWN. 1971. Potassium and chloride activities in identifiable *Aplysia* neurons. *Fed. Proc.* **30:**255.

LEV, A. A. 1964. Determination of activity and activity coefficients of potassium and sodium ions in frog muscle fibres. *Nature (Lond.)*. **201:**1132–1134.

MILLECCHIA, R., and A. MAURO. 1969. The ventral photoreceptor cells of *Limulus*. III. A voltage clamp study. *J. Gen. Physiol.* **54:**331–351.

NEILD, T. O., and R. C. THOMAS. 1974. Intracellular chloride activity and the effects of acetylcholine in snail neurones. *J. Physiol. (Lond.)*. **242:**453–470.

ORME, F. W. 1969. Liquid ion-exchanger microelectrodes. *In* Glass Microelectrodes. M. Lavallee, O. Schanne, and N. C. Hebert, editors. John Wiley & Sons, New York.

OTTOSON. D. 1964. The effect of sodium deficiency on the response of the isolated muscle spindle. *J. Physiol. (Lond.)*. **171:**109–118.

OWEN, J. D., H. M. BROWN, and J. H. SAUNDERS. 1975. Chloride activity in the giant cell of *Aplysia*. *Biophys. J.* **15:**45 a.

SAUNDERS, J. H., and H. M. BROWN. 1975. Determination of intracellular chloride in *Balanus eburneus* photoreceptor. *Biophys. J.* **15:**323 a.

SOROKINA, Z. A. 1964. The activity of potassium and sodium ions in the protoplasm of striated muscle fibers in the frog. *Byull. Eksp. Biol. Med.* **12:**17–21.

STRICKHOLM, A., and B. G. WALLIN. 1965. Intracellular chloride activity of crayfish giant axons. *Nature (Lond.)*. **208:**790–791.

THOMAS, R. C. 1969. Membrane current and intracellular sodium changes in a snail neurone during extrusion of injected sodium. *J. Physiol. (Lond.)*. **201:**495–514.

THOMAS, R. C. 1970. New design for sodium-sensitive glass micro-electrode. *J. Physiol. (Lond.)*. **210**:82–83P.

THOMAS, R. C. 1972. Intracellular sodium activity and the sodium pump in snail neurones. *J. Physiol. (Lond.)*. **220**:55–71.

WALKER, J. L. 1971. Ion specific liquid ion-exchanger microelectrodes. *Anal. Chem.* **43**:89A–92A.