Intracellular Na⁺, K⁺, and Cl⁻ Activities in Balanus Photoreceptors

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ABSTRACT Ion-sensitive microelectrodes were used to measure intracellular activities \( a_i^{Na}, a_i^{K}, \) and \( a_i^{Cl} \) 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 \( a_i^{K} \) 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 \( a_i^{K} \). 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 \( a_i^{K} \) 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 $a_x^i$ were obtained as $a_K^i$ 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; MgCl₂, 12; CaCl₂, 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 ($a_x^i$) 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 $a_x^i$. $a_K^i$ was determined in five cells with microelectrodes filled with 3 M NaCl and $a_{Cl}^i$ 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 ($V_i - E_m$) after penetration reflects a decrease in $a_{Na}^\text{in}$. This difference became greater during illumination which indicates $a_{Na}^\text{in}$ 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 ($V_i$) 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 ($10^{14}$ ohms); $c$, differential amplifier for cancellation of potential drop across recording microelectrode during current pulses.

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 ($k_{i,Na}$) 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 $a_{Na}^\text{in}$ 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 $a_{Cl}^\text{in}$ 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,
For example $k_{\text{Cl, lactate}}$ was about 0.20 and $k_{\text{Cl, HCO}_3}$ 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 ($k_{\text{Na, K}}$) 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 $k_{\text{Na, K}}$ for different electrodes ranged between $5 \times 10^{-3}$ to $1 \times 10^{-3}$. A small error was possible in calculating $a^{i}_{\text{Na}}$ for certain of the Na⁺ electrodes, since intracellular $a^{i}_{\text{Na}}$ is relatively high compared to the level of intracellular Na⁺. Therefore, the $a^{i}_{\text{Na}}$ measurements were corrected routinely by an appropriate amount depending upon the $k_{\text{Na, K}}$ 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 $10^{13}$ 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 ($V_{j}$) with respect to this reference in the external saline. When both electrodes were inside the cell the difference in potential between the two electrodes ($V_{j} - E_{m}$) was used to calculate the internal ion activity ($a^{i}_{x}; x = \text{Na}^{+}, \text{K}^{+}, \text{or Cl}^{-}$) from the relation:

$$a^{i}_{x} = a^{e}_{x} \cdot \exp \left( \frac{V_{j} - E_{m}}{m} \right) - k_{x y} a^{i}_{y}$$

where $V_{j}$ is the potential of the ion electrode in the external saline with primary ion activity $a^{e}_{x}; V_{j}$ 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 $x; k_{x y}$ 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 5 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 (\(a_{\text{Na}}^0 = 315\) 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 \((V_i - E_m)\) values used to calculate \(a_{\text{Na}}^i\) 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 potential resumed its earlier value after the light flashes. The slight increase in \((V_i - E_m)\) reflects a small increase in \(a_{\text{Na}}^i\). 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 \((V_i - E_m)\) was 34 mV which yields an \(a_{\text{Na}}^i\) of 13 mM (eq. 1). However, the potential difference recorded after 6 m of illumination \((V_i - E_m)_L\) was 38 mV which yields a value of 15.4 mM for \(a_{\text{Na}}^i\); thus, there was a net increase in \(a_{\text{Na}}^i\) of 2-3 mM during the exposure to light in this cell. A similar exposure to light in some other cells increased \(a_{\text{Na}}^i\) by as much as 5 mM.

Final values of \(a_{\text{Na}}^i\) 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 \(a_{\text{Na}}^i\) 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 exchange 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 \(a_{\text{Na}}^i\) by cellular pumping mechanisms could also be involved but the time constants of \(a_{\text{Na}}^i\) after penetration with Na\(^+\) electrodes were generally at least twice as long as those observed for changes in \(a_{\text{Na}}^i\) 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 (\(a_{\text{Na}}^i\)) 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 ($a'_{Na}$) MEASURED IN 13 DIFFERENT CELLS WITH 13 DIFFERENT Na⁺-SENSITIVE ELECTRODES

| Cell | $a'_{Na}$ | $E_{Na}$ | $E_m$ | t (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      |
| $\bar{t}$ | 28    | +64      | -40   | 42      |
| SD (±) | 13.6     | 14.4     | 3.5   | 18      |

Data are ranked from the lowest to highest value of $a'_{Na}$. For example, similar values of $a'_{Na}$ 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 $a'_{Na}$ 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 of a smaller KCl-filled pipette (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 $a'_{Cl}$ 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 $a'_{Cl}$ 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 $E_{\text{Cl}}$ and $E_{\text{m}}$. The cell by cell differences between $E_{\text{Cl}}$ and $E_{\text{m}}$ 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 $V_f$ and $E_{\text{m}}$. There was little evidence of any change in $a'_{\text{Cl}}$ when the cell was illuminated for the same period of time required to elicit a change in $a'_{\text{Na}}$ (about 5 min). Therefore, even though Cl$^{-}$ seems to be at equilibrium in the dark-adapted state, there is no immediate change in $a'_{\text{Cl}}$ when the membrane potential is altered with illumination.

**Internal K$^+$ Activity**

Steady-state values for $a'_{\text{K}}$ 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 $a'_{\text{K}}$ were obtained as soon as steady values were reached so that $a'_{\text{K}}$ 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 $a'_{\text{K}}$ 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 $E_{\text{K}}$ were only 12 mV different between each extreme. The average values of $a'_{\text{K}}$ and $E_{\text{K}}$ shown at the bottom of the table were 120 mM and -77 mV, respectively. Unlike $E_{\text{Cl}}$, $E_{\text{K}}$ was consistently more negative than $E_{\text{m}}$: the difference between $E_{\text{K}}$ and $E_{\text{m}}$ 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 ($E_{\text{m}}$) in these cells is comparable to

| Cell | $a'_{\text{Cl}}$ (mM) | $E_{\text{Cl}}$ (mV) | $E_{\text{m}}$ (mV) | $E_{\text{Cl}} - E_{\text{m}}$ (mV) |
|------|----------------------|---------------------|---------------------|----------------------------------|
| 1    | 94                   | -32                 | -28                 | -4                               |
| 2    | 76                   | -38                 | -40                 | +2                               |
| 3    | 72                   | -39                 | -40                 | +1                               |
| 4    | 60                   | -43                 | -40                 | -5                               |
| 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                               |
| $\bar{x}$ | 65              | -42                 | -41                 | -0.1                             |
| SD ($\sigma$) | 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 ($E_{\text{Cl}}$) calculated from $E_{\text{Cl}} = 58 \log_{10} (a'_{\text{Cl}}/325 \text{ mM})$; 325 mM was the average value of barnacle saline from several independent determinations with Ag/AgCl electrodes. The difference between the mean values of $E_{\text{Cl}}$ and $E_{\text{m}}$ shown in column 5 was not significant ($t$-test).
**Table III**

**INTERNAL POTASSIUM ACTIVITY ($a'_K$) MEASUREMENTS FROM 11 CELLS WITH 11 DIFFERENT $K^+$-SENSITIVE ELECTRODES**

| Cell | $a'_K$ (mM) | $E_K$ (mV) | $E_m$ (mV) | $E_m - E_K$ |
|------|-------------|------------|------------|-------------|
| 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         |

SD (±) 17.3 4.2 3.0 4.6

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

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

that in which $a'_Na$ and $a'_Cl$ 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.

### $a'_K$ 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 ($\alpha$) can be calculated:

$$\exp \frac{E_mF}{RT} = \frac{\alpha \cdot M}{a'_K} + \frac{1 - \alpha}{a'_K}$$

where $M = a'_Na + a'_K$ and $F$, $R$, and $T$ have their usual meanings. This calculation of $\alpha$ rests on the assumptions that (1) $a'_K$ is relatively constant at all concentrations of external potassium, (2) that the value of $a'_K$ can be specified...
from the relation between $E_m$ and $a_{0m}^i$, 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 $a$ from the $E_m$ vs. $a_{0m}^i$ 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 $a_{0m}^i$ the cell was returned to normal saline as indicated by multiple data points at $a_{0m}^i = 5.5$ mM. As the $a_{0m}^i$ was increased the membrane was depolarized; the maximum rate of membrane potential change per decade $a_{0m}^i$ 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 $p_{Na}:p_K$ value of 0.13 was obtained for this cell. This should be considered a first approximation at best since data obtained from measurements of $a_{0m}^i$ with K+-sensitive electrodes under the same experimental conditions revealed that rather large changes in $a_{0m}^i$ 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 $a_{0m}^i$ was increased the $a_{0m}^{iK}$ also increased significantly. The same was true for the other cell which had a higher initial $a_{0m}^i$ in normal saline (solid squares). The increase was especially apparent for changes in $a_{0m}^i$ from 5.5 mM to 160 mM; at higher values of $a_{0m}^i$, $a_{0m}^{iK}$ remained relatively constant. Thus, as $a_{0m}^i$ was increased to 160 mM, the value of $a_{0m}^{iK}$ approached the same value. From these data, assumption (1) that $a_{0m}^{iK}$ remains constant as $a_{0m}^i$ 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 $a_{0m}^{iK}$ obtained from Fig. 2 overestimates the true $a_{0m}^{iK}$. The intersection of the curve
FIGURE 3. A, internal K⁺ activity (aK) measured with liquid ion-exchanger microelectrodes as the external K⁺ activity (a°K) was raised in the external saline. Two cells; solid circles same cell as Fig. 2. B, relationship between membrane potential (Em) and (EK) 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 EK were obtained when Em and aK had attained steady-state values after a change in a°K.

In Fig. 2 with 0-membrane potential corresponds to an aK of 245 mM. The value of aK measured directly with the K⁺ electrode in Fig. 3A was about 150 mM at the same a°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 Em and experimental values of EK at different a°K's is examined. This relationship is shown in Fig. 3B for four different cells. Data from the cell shown in Figs. 2 and 3A are represented by solid circles. The solid line represents a perfect correspondence between Em and EK. As the a°K was increased the correspondence between Em and EK was much better than would have been predicted from Fig. 2 or α calculated from eq. 1. The data in Fig. 3B 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 Em = EK. A revised estimate of pNa: pK (α') can be obtained from data based on directly measured values of aK as a°K was varied:

\[
\alpha' = \exp \left( \frac{E_m F (5 + a'_K - a^o_K)}{RT} \right) a^o_{Na}
\]

where aK and a°K were measured directly (Figs. 2 and 3A) and a^o_{Na} = 320 - a°K; 5
is the product of \( a'_{Na} \) and \( \alpha \). This product is an upper limit based on the highest value of \( a'_{Na} \) obtained in this investigation (Table I) and the average \( \alpha \) from a series of experiments using eq. 2. The estimate of \( pNa : pK \) calculated from (3) at the resting potential \( (E_m = -40 \text{ mV}) \) is considerably less than that obtained previously from eq. 2 \((0.04 \text{ vs. } 0.13)\), but the ratio varies depending on \( a'_{K} \) (and/or \( E_r \)). Table IV, column 3 shows \( \alpha' \) calculated for four different membrane potential values. The negative values at \( E_m = -8 \text{ mV} \) 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. 5 B, i.e., for the smaller increases of \( a'_{K} \) the membrane potential was more positive than \( E_K (E_m > E_K) \). This would yield positive \( \alpha' \) values. But at greater \( a'_{K} \)’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 \( \alpha' \) values. This reversal of \( E_m \) with respect to \( E_K \) as \( a'_{K} \) 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., \( pK > pCl \gg pNa \). If experimental values of \( a'_{K} \) at different \( a'_{K} \)’s and the experimentally determined mean value of \( a'_{Na} \) (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

| \( E_m^* \) | \( E_K \) | \( a' \) | \( E_m^\dagger \) |
|-------------|-----------|---------|---------------|
| -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 \( a'_{K} \) where a \( Cl^- \) permeability term has been included.

* Fig. 2 solid data points.
† Eq. 3 in text.
§ Calculated from measured values of \( a'_{K}, a'_{Na}, a'_{Cl}, \) and \( pK : pNa : pCl = 1:0.04:0.3 \).
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 $a'$ (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 $a'_{Na}$, 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 McLoughlin, 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 $a'_{Na}$, 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.

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