DEPENDENCE OF CELLULAR POTENTIAL ON IONIC CONCENTRATIONS

Data Supporting a Modification of the Constant Field Equation

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ABSTRACT The resting potential in the squid axon has been measured at various concentrations of Cl, K, Na, and Ca ions. The results of these measurements are compared with the Goldman-Hodgkin-Katz (GHK) equation and a modified constant field equation. This modified equation was derived by including currents carried by divalent ions and the effects of the unstirred layer and the periaxonal space. It is shown that, although the GHK equation can fit the V vs. $[K]_o$ data well, it has difficulty explaining the observed dependence of V on $[Na]_o$ when the axon is bathed in K-free artificial sea water. The use of the modified constant field equation removes this difficulty.

INTRODUCTION

The Goldman-Hodgkin-Katz (GHK) equation is one of the most widely used equations in electrobiology. This equation not only provides a theoretical basis to explain the relationship between the membrane potential and the ionic concentration gradients, but it also allows one to calculate the permeability ratios of the ions, using measurements of potential. It has long been recognized, however, that the GHK equation is an approximation that involves many idealized assumptions (Hodgkin and Katz, 1949; Tasaki, 1968; Schwartz, 1971). Some of these assumptions, such as constant field and ion independence, may be hard to justify in a biological system. Furthermore, the derivation of the GHK equation does not consider currents carried by divalent ions, and the effect of the "unstirred layer" outside the membrane has been ignored. Thus, it is important to see how well the GHK equation can be applied to different biological systems.

In this paper, I report results of measurements of the resting potential in squid axons as a function of the external concentrations of Cl, Na, K, and Ca ions, and of the internal concentration of Cl ions. I show that the GHK equation can fit the observed data only partially. Some of the ionic dependence of the resting potential is difficult to explain.

One wonders whether this observed difficulty of the GHK equation is due mainly to the deficiency of the constant field approach, or to the failure of other simplifying assumptions involved in the derivation of the GHK equation. In this study, the latter case has been investigated. Without abandoning the constant field assumption, I have suggested a modification of the GHK equation to include the effects of currents carried by divalent ions and the effects of the unstirred layer and the periaxonal space. This modified constant field equation seems to fit the experimental data satisfactorily.

METHODS AND MATERIALS

The biological sample used in this study is the giant axon of the squid *loligo pealei*, supplied by the Marine Biological Laboratory, Woods Hole, MA, where the experiments were conducted. Use of the squid axon to study the resting potential provides several advantages: (*a*) the structure of the giant axon is simple, so that complications due to transport among multiple compartments are eliminated; (*b*) the giant axon is large, so that an axial electrode can be inserted and damage to the membrane can be avoided; (*c*) the giant axon can be perfused internally, so that both the internal and external ionic concentrations can be changed; and (*d*) because of the large cell size and the low impedance of the axial electrode, the resting potential of the squid axon usually is very stable and varies little (at most a few millivolts) from axon to axon.

The design of the experimental apparatus was similar to those used in the laboratories of Narahashi (Wu and Narahashi, 1973) and Tasaki (Tasaki et al., 1965). The axon was perfused through a single glass cannula; the technique is a modification of that of Tasaki (Tasaki et al., 1965). The resting potential was measured by using an axial internal electrode and an external reference electrode. These electrodes are glass capillaries filled with 3 M KCl solution that was in contact with an Ag-AgCl electrode. Each of these electrodes was connected to a high input impedance operational amplifier (Analog Devices, Inc., Norwood, MA, model AD506), the differential output of which was recorded by digital electronics.

The junction potentials of the electrode system are corrected by using an experimental arrangement similar to those used by Baker et al. (1964). That is, before it was inserted into an axon, the internal electrode was first placed into a reservoir of 0.6 M KCl solution that was connected to a pool of artificial sea water by a salt bridge (made with artificial sea water [ASW] in 3% agarose). The reference electrode was in contact with the pool of ASW through another salt bridge. The differential output of the two electrodes is defined as V_{offset}, which supposedly contains all junctional

TABLE I
COMPOSITION OF ARTIFICIAL SEA WATER (ASW)

ASW	KCl	NaCl	$CaCl_2$	$MgCl_2$	HEPES buffer	NaOH	Choline chloride	CsOH
	mM	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*
Normal	10	420	20	50	5	5	0	0
K-free	0	420	20	50	5	5	0	0
K and Na-free	0	0	20	50	5	0	430	3
K and Mg-free	0	470	50	0	5	4	0	0
High Ca	0	50	250	0	5	5	20	0
Variable $[K]_o$	same as normal except part of NaCl is replaced by KCl							
Variable $[Na]_o$	same as K-free except part of NaCl is replaced by choline chloride							
Variable $[Cl]_o$	same as K and Mg-free except part of NaCl is replaced by Na propionate							
Variable $[Ca]_o$	same as high Ca except part of $CaCl_2$ is replaced by choline chloride							

potentials. When the internal electrode was inserted into the axon, the newly recorded differential potential was denoted V. The experimental value of the resting potential was taken as the difference between V and V_{offset}. As a standard procedure, V_{offset} was determined again at the end of each experiment. It usually stayed within 2 mV of the initial value.

Because one of the most important factors that affect the measurement of a resting potential is leakage, all axons used in this study were dissected carefully so that leakage was at a minimum. At the beginning of each experiment, the resting potential had to be at least −60 mV and the magnitude of the action potential not less than 100 mV. Otherwise, the axon would be rejected.

The composition of the ASW is given in Table I. The control internal perfusion solution (IPS) was composed of 360 mM KF, 40 mM K-phosphate, 50 mM NaF, and 200 mM sucrose. This solution is similar to that used by Tasaki et al. (1965). The ASW and IPS were buffered and the pH adjusted to 7.9 and 7.3, respectively. The activity coefficients of K and Na in the various solutions were estimated from data compiled by Robinson and Stokes (1959). γ_K and γ_{Na} in ASW were taken as the average activity coefficients ($\gamma_\pm$) of KCl and NaCl, respectively, in solutions of the same ionic strength as the ASW, while γ_K and γ_{Na} in IPS were taken as the $\gamma_\pm$ of KF and NaF, respectively. The values I used for the activity coefficients were $\gamma_K = 0.632$ and $\gamma_{Na} = 0.670$ for the ASW; $\gamma_K = 0.676$ and $\gamma_{Na} = 0.641$ for the internal solution. For the purpose of calculation, the activities of Na and K inside an intact axon were assumed to be the same as in the normal IPS. Except for those specified, the experiments were done at a temperature of 5°C.

When the axon was perfused with a solution that contained a different concentration of electrolyte, the resting potential of the axon changed quickly and often reached a new, stable value within minutes. The time course of the change of potential usually simulated an exponential curve. The asymptote of the potential curve was chosen to be the final value of the resting potential. I often recorded two asymptotes in each measurement, one determined by a change from a high concentration to a low concentration, and another by a change from a low concentration to a high concentration. These two values usually agreed to within a few millivolts.

RESULTS AND DISCUSSION

If one adopts the convention that the resting potential, V, is measured from the inside of the cell against the outside, the GHK equation gives

$$V = RT/F \ln\left[\frac{P_K(K)_o + P_{Na}(Na)_o + P_{Cl}(Cl)_i}{P_K(K)_i + P_{Na}(Na)_i + P_{Cl}(Cl)_o}\right], \quad (1)$$

where R, T, and F are the universal gas constant, temperature (in Kelvin), and Faraday's constant, respectively; P_K, P_{Na}, and P_{Cl} are the relative membrane permeabilities of K, Na, and Cl; (K), (Na), and (Cl) are the activities of K, Na, and Cl; subscript o denotes the outside of the cell, and subscript i denotes the inside of the cell. In this paper, (S) means activity of ion S, while [S] means concentration of ion S. $(S) = \gamma_S[S]$. In most biological studies, however, a simplified form of the GHK equation often is used, i.e.,

$$V = RT/F \ln\left[\frac{(K)_o + b(Na)_o}{(K)_i + b(Na)_i}\right], \quad (2)$$

where $b = P_{Na}/P_K$. This "simplified GHK equation," proposed by Hodgkin (1958), has the advantage of containing only one unknown parameter, b. If the ionic activities are known, the permeability ratio can be determined simply by measuring V.

Dependence of the Cellular Potential on Concentrations of Cl

To determine whether the simplified form of the GHK equation (Eq. 2) can be used safely, I have examined the effects of $[Cl]_i$ and $[Cl]_o$ on the cellular potential. It is stated often in the literature that the Cl terms in Eq. 1 can be dropped, because Cl ion distributes passively across the cell membrane, and hence the net Cl current is zero. This argument cannot be applied to the experiments with squid axons because, during internal perfusion, both $[Cl]_o$ and $[Cl]_i$ can be varied at will, and Cl is not at equilibrium. The justification for reducing Eq. 1 into Eq. 2, therefore, must rest on experimental observation.

Measurements of resting potential at two different $[Cl]_i$ under various ionic conditions are summarized in Table II. Here $[Cl]_i$ is changed from 40 mM (physiological concentration) to 0 mM. It is clear from the observed data that the $[Cl]_i$ has no effect on the resting potential. Changes in $[Cl]_o$, also, do not change the resting potential signifi-

TABLE II
AVERAGE RESTING POTENTIAL AT DIFFERENT $[Cl]_i$

$[K]_o$	$[Na]_o$	$[Cl]_o$	$[K]_i$	$[Na]_i$	$[Cl]_i$	Resting potential*
mM	*mM*	*mM*	*mM*	*mM*	*mM*	*mV*
10	425	570	400	50	40	60.0 ± 2.1 (7)
10	425	570	400	50	0	60.3 ± 1.7 (6)
0	425	560	400	50	40	65.5 ± 2.1 (5)
0	425	560	400	50	0	67.1 ± 1.4 (6)
0	0	570	400	50	0	70.6 ± 2.0 (8)

*Mean ± standard deviation (number of axons).

cantly, regardless of whether $[Cl]_o$ changes with $[Na]_o$ or not (Table III). Therefore, the simplification of the GHK equation as proposed by Hodgkin (i.e., Eq. 2) is valid for the squid axon and will be used in this paper.

Dependence of the Resting Potential on $[K]_o$

The first experimental test of the GHK equation was to examine how the resting potential changes as a function of $[K]_o$ (Hodgkin and Katz, 1949; Curtis and Cole, 1940). Hodgkin (1958) has shown that the simplified GHK equation can qualitatively fit the V vs. $(K)_o$ data if a value for b of ~0.01 is taken. Success of similar fitting also has been reported for frog nerve (Huxley and Stämpfli, 1951), insect muscle (Wood, 1957), and toad egg (Maéno, 1959).

To examine in detail the dependence of the resting potential on $(K)_o$, I repeated the measurements of Curtis and Cole (1940) in a number of axons, both intact and internally perfused (see Fig. 1). My results confirm the earlier studies. The solid line in Fig. 1, a theoretical curve based on the simplified GHK equation in which b is estimated to be 0.0563, fits the data satisfactorily.

One feature of the experimental data in Fig. 1 is that as $(K)_o$ is reduced to near zero, V deviates greatly from the Nernst potential of K ion and approaches a constant value.

TABLE III
RESTING POTENTIAL AT DIFFERENT $[Cl]_o$

Axon No.	$[K]_o$	$[Na]_o$	$[Cl]_o$	Resting potential
	mM	*mM*	*mM*	*mV*
78–18	0	473	573	−66.3
	0	473	100	−68.8
	0	104	100	−64.9
78–19	0	473	573	−62.6
	0	473	285	−64.9
	0	473	100	−63.4
	0	104	100	−61.9

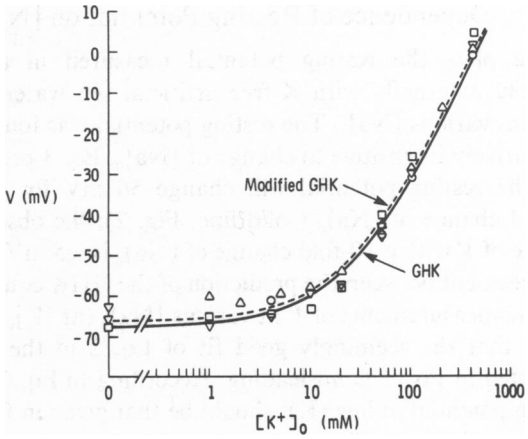

FIGURE 1 Resting potential vs. external concentration of K ion. Symbols represent data from different axons: △, axon 77-80; ▽, axon 78-44; ○, axon 78-45; □, axon 78-68. Axon 77-80 was an intact axon, and the rest were perfused internally with normal IPS. Solid curve (labeled GHK) is a fitting of Eq. 2 with $b = 0.0563$. Broken curve (labeled modified GHK) is a fitting of Eq. 10 with $b = 0.01$ and $c = 0.053$.

This behavior can be explained by Eq. 2, in which

$$\lim_{(K)_o \to 0} V = \frac{RT}{F}\left[\frac{b(Na)_o}{(K)_i + b(Na)_i}\right]. \quad (3)$$

Accordingly, V should approach a constant independent of $(K)_o$ at low $(K)_o$. This explanation, however, contains the untested prediction that, at low $(K)_o$, V would be directly proportional to the logarithm of $(Na)_o$. This important prediction has not been examined experimentally in previous studies. I decided to test it in the squid axon.

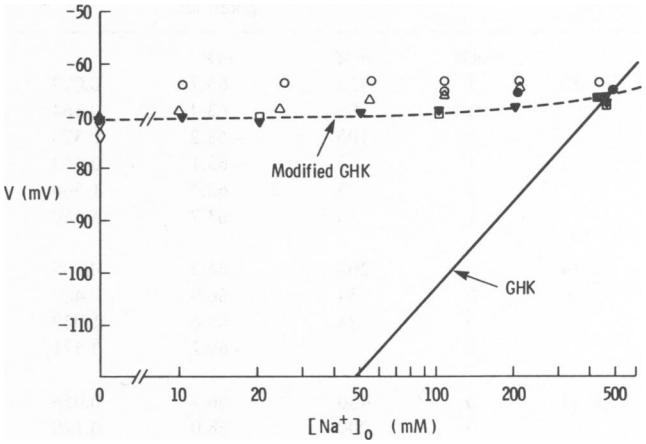

FIGURE 2 Resting potential vs. external concentration of Na ion. Symbols represent different axons: ○, axon 77-83; ●, axon 78-13; △, axon 78-14; ▼, axon 79-11; □, axon 79-14; ◇, axon 80-41; ▲, axon 80-48; ▽, axon 80-49; ■, axon 81-8. Axons 77-83, 78-13, 78-14, 79-11, and 79-14 were intact axons. Axons 80-41, 80-48, 80–49, and 81-8 were perfused with normal IPS. All axons were bathed in K-free ASW at a temperature of 5°C, except for axon 77-83, which was bathed in 1 mM K ASW at a temperature of 21°C. The solid curve is a fit by Eq. 2 with $b = 0.0563$. The broken curve is a fit by Eq. 10 with $b = 0.01$ and $c = 0.053$.

Dependence of Resting Potential on $[Na]_o$

Fig. 2 plots the resting potential measured in axons perfused externally with K-free artificial sea water that contains various $[Na]_o$. The resting potential was found to be relatively insensitive to change of $[Na]_o$. Eq. 3 predicts that the resting potential will change 56 mV for every 10-fold change of $(Na)_o$ (solid line, Fig. 2); the observed change of V with a 10-fold change of $(Na)_o$ is <5 mV. The disagreement between the prediction of the GHK equation and the measurements of V at various $[Na]_o$ (at $[K]_o = 0$) shows that the seemingly good fit of Eq. 2 to the data presented in Fig. 1 is misleading. According to Eq. 2, the resting potential at low $(K)_o$ should be that given in Eq. 3; this is not the case (Fig. 2).

One may argue that the permeability ratio could be a function of $(Na)_o$, and if one allowed b to vary, Eq. 3 might fit the data in Fig. 2. There are several difficulties with this argument. First, to make Eq. 3 fit the data in Fig. 2, b must vary widely with change of $(Na)_o$ (see Table IV). This means that the values of P_{Na}/P_K determined from the measurements of potential (using the GHK equation) are arbitrary and depend on the value of $(Na)_o$ chosen. Second, fitting Eq. 3 to the data in Fig. 2 requires that P_{Na}/P_K vary inversely with $[Na]_o$ (see Fig. 3). When $[Na]_o$ approaches zero, P_{Na}/P_K would have to approach infinity! Third, when the GHK equation was used to fit the data in Fig. 1, "b" was taken as a constant. If b is regarded as a variable that depends on the ionic activities, there will be a discrepancy between the fitting of the data in Fig. 1 and that in Fig. 2.

TABLE IV
CALCULATED PERMEABILITY RATIO

Axon No.	$[K]_o$	$[Na]_o$	Resting potential	P_{Na}/P_K
	mM	*mM*	*mV*	
77–83	1	425	−63.7	0.077
	1	205	−63.4	0.164
	1	105	−63.2	0.328
	1	55	−63.1	0.653
	1	25	−63.4	1.566
	1	10	−63.7	5.380
78–14	0	204	−64.3	0.138
	0	54	−66.9	0.487
	0	24	−68.6	1.089
	0	9	−69.2	3.571
79–11	0	450	−66.7	0.056
	0	200	−68.0	0.120
	0	100	−68.8	0.236
	0	50	−69.5	0.471
	0	20	−70.8	1.207
	0	10	−69.8	2.980
	0	0	−70.8	∞

The value P_{Na}/P_K was calculated from Eq. 2 by assuming V equal to the measured resting potential. All axons are intact axons. For axon 77–83, the experiment was done at 21°C; for axons 78–14 and 79–11, the experiments were done at 5°C.

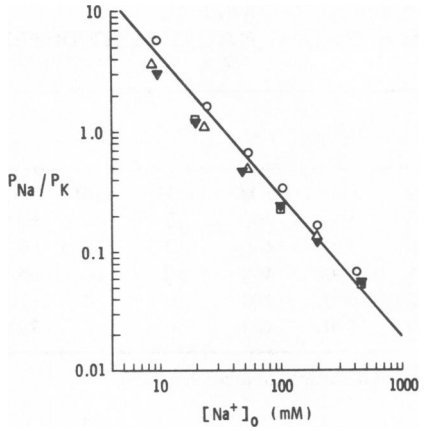

FIGURE 3 The calculated values of P_{Na}/P_K obtained by fitting the data presented in Fig. 2 using Eq. 2, to plot them as a function of $[Na]_o$. The solid line shows that P_{Na}/P_K is inversely proportional to $[Na]_o$. Symbols have the same meanings as in Fig. 2.

Because of these difficulties, I do not think the GHK equation can be defended solely by arguing that b is not a constant. In fact, regardless of whether b is a constant or not, the GHK equation cannot explain why V remains finite when $(K)_o$, $(Na)_o$, and $(Cl)_i$ all approach zero.

Modification of the GHK Equation

Consideration of Divalent Ions. To account for the finite resting potential observed when the axon is bathed in K- and Na-free ASW, one must modify the GHK equation to include the effects of currents carried by ions other than Na, K, and Cl. In the experiment in which the external Na^+ ions are replaced by choline ions, one may suspect that choline ions may enter the cell and could thus affect the membrane potential. However, I think this effect of choline is relatively unimportant. First, the permeability of choline to the axon membrane is known to be very small. For example, Hodgkin and Huxley (1952) used choline to replace external Na^+ ions in their squid axon experiments, and they found that the inward current carried by choline was negligible. Second, I observed that when Na^+ is replaced by choline, the action potential is totally abolished, again indicating that choline is much less permeable than Na^+. Third, to explain the data shown in Fig. 2, the permeability of choline to the squid axon membrane would be required to equal the permeability of Na^+, because V does not vary under a one-to-one ion exchange. This requirement clearly cannot be satisfied. Fourth, no significant change of the resting potential was observed when I diluted the choline artificial sea water with 12% glycerol solution. Therefore, I conclude that the contribution of the choline ions to the resting potential must be very small.

However, there are other ions in the sea water that may have a more important effect on the nerve cell. One of the most important ions in biological systems is Ca ion, which

is known to control many biological functions. I suspect that Ca ions may play a significant role in determining the resting potential. Indeed, I found experimentally that the resting potential in squid axons is sensitive to the concentration of Ca ions. A 10-fold change of $[Ca]_o$ changes the resting potential by ~12 mV (Table V) (which is equivalent to a change of 24 mV for a monovalent ion).

Following procedures similar to those used by Hodgkin and Katz (1949), I have derived a modified constant field equation that takes into account the contributions of currents carried by divalent ions (Ca and Mg). The derivation is given in the Appendix. To simplify the derivation, I assumed that the net current carried by Cl ions is small and thus can be left out (as it is in the simplified GHK equation). The internal activity of Ca also is taken as zero, since the concentration of free Ca ion inside the cell is known to be very small (Baker, et al., 1971). This new constant field equation is shown to be

$$V = RT/F \cdot \ln\left[\frac{P_K(K)_o + P_{Na}(Na)_o + 4P_{Ca}(Ca)_o + 4P_{Mg}(Mg)_o}{P_K(K)_i + P_{Na}(Na)_i + 4P_{Mg}(Mg)_i}\right]. \quad (4)$$

The term for the divalent ion in the denominator is probably insignificant, because $P_K(K)_i$ is much larger than $P_{Mg}(Mg)_i$ in most biological systems. The terms for divalent ions in the numerator, on the other hand, cannot be ignored, because $(K)_o$ generally is small. In fact, the terms for divalent ions dominate when $(K)_o$ approaches zero. Thus, Eq. 4 may be simplified further to become

$$V = RT/F \cdot \ln\left[\frac{P_K(K)_o + P_{Na}(Na)_o + 4P_{Ca}(Ca)_o + 4P_{Mg}(Mg)_o}{P_K(K)_i + P_{Na}(Na)_i}\right]. \quad (5)$$

Consideration of the effects of divalent ions on the resting potential is not totally new. For example, Sperelakis (1979) discussed the effects of Ca^{++} on the resting potential of cardiac cells using a modified version of the Goldman equation that includes Ca^{++} terms. The derivation of this modified Goldman equation was attributed to Dr. D. E. Goldman (Sperelakis, 1979). However, unlike Eq. 5, the modified constant field equation used by Sperelakis is somewhat cumbersome and contains a nonlinear term in the logarithm and thus is difficult to use for fitting the V vs. ionic concentration data. This difficulty is removed in the derivation of my modified constant field equation by using a proper linearized approximation.

TABLE V
RESTING POTENTIAL AT DIFFERENT $[Ca]_o$

Axon No.	$[Ca]_o$	Resting potential
	mM	*mV*
78–21	10	−71.4
	20	−68.8
	50	−64.2
	100	−60.2
	250	−54.9
78–22	10	−72.6
	20	−69.3
	50	−63.5
	100	−60.0
	250	−54.6

Sperelakis (1979) maintained that the Ca^{++} effect on the resting potential is very small and hence can be ignored. This could be so in the cardiac system because the $[Ca]_o$ is small there. In general, however, as shown by the data in Table V, the contribution of divalent ions to the resting potential cannot be ignored when $[Ca]_o$ is high, which is the case for marine animals.

Consideration of the Effects of the Unstirred Layer and the Periaxonal Space. In the derivation of the GHK equation, it was assumed that the ionic concentration at the surface of the membrane is directly proportional to the ionic concentration of the external solution, i.e., $(K)_{\text{outer surface of membrane}} = \beta_K(K)_o$. Obviously, this assumption cannot hold when the axon is bathed in K-free artificial sea water. First, there is an unstirred layer of water outside of the membrane (Dainty, 1965), which forms a diffusion barrier and thus maintains an effective [K] somewhere between $[K]_i$ and $[K]_o$ at the outside of the membrane. Second, the membrane of the squid axon is separated from the external fluid by a "periaxonal space" (Adelman and Palti, 1972). Because $[K]_i$ is normally high, the K ions diffusing from the axon could be trapped temporarily in the periaxonal space and make the effective $(K)_o$ sensed by the membrane different from that of the external fluid. To account for these effects, the ionic activities in Eq. 5 should be identified with the effective ionic activities at the regions adjacent to the membrane surface, which can be estimated from Fick's law. For example, suppose the combined permeability of the unstirred layer and the opening between the periaxonal space and the external fluid is P_{DS} for S ions (S could be K, Na, etc.); the flux of S is

$$J_S = P_{DS}([S]_{\text{outside membrane}} - [S]_o)$$

or

$$[S]_{\text{outside membrane}} = \frac{J_S}{P_{DS}} + [S]_o. \quad (6)$$

From the above relation, one can see that $[S]_{\text{outside membrane}}$ differs from $[S]_o$ when J_S/P_{DS} is nonnegligible compared with $[S]_o$. Because J_S equals the current carried by S ions across the membrane, J_S is proportional to P_S (the membrane permeability of S ion). This means that the effects of the unstirred layer and the periaxonal space are important for those ions (*a*) to which the membrane is highly permeant and (*b*) that have low concentration in the

bathing fluid. For practical purposes, only the K ions at the external side seem to satisfy these criteria. Therefore, to account for the effects of the unstirred layer and the periaxonal space, one must change $(K)_o \rightarrow (K)_o^{effective}$ in Eq. 5, with

$$(K)_o^{effective} = \gamma_K [K]_{outside\ membrane}. \quad (7)$$

Because

$$(K)_o = \gamma_K [K]_o.$$

Eq. 6 implies

$$(K)_o^{effective} = \frac{\gamma_K J_K}{P_{DS}} + (K)_o. \quad (8)$$

Eq. 5 then should become

$$V = RT/F \cdot \ln\left[\frac{P_K(K)_o + P_{Na}(Na)_o + 4P_{Ca}(Ca)_o + 4P_{Mg}(Mg)_o + \frac{\gamma_K J_K P_K}{P_{DK}}}{P_K(K)_i + P_{Na}(Na)_i}\right]. \quad (9)$$

Eq. 9 is consistent with the usual GHK equation, since if one ignores the effects of divalent ions, the unstirred layer, and the periaxonal space (i.e., setting $P_{Ca} = P_{Mg} = J_K = 0$), Eq. 9 will be reduced to Eq. 2, the simplified GHK equation.

Fitting the Modified GHK Equation to Experimental Data

To compare the modified GHK equation with the original GHK equation, Eq. 9 can be rewritten as

$$V = RT/F \ln\left[\frac{(K)_o + b(Na)_o}{(K)_i + b(Na)_i} + c\right], \quad (10)$$

where $b = P_{Na}/P_K$ and

$$c = \frac{4P_{Ca}(Ca)_o + 4P_{Mg}(Mg)_o + \frac{\gamma_K J_K P_K}{P_{DK}}}{P_K(K)_i + P_{Na}(Na)_i}.$$

Because the J_K term is significant only when $(K)_o$ is small, J_K can be regarded as roughly proportional to $(K)_i$. As a first-order approximation, c may be regarded as a constant independent of $(K)_o$ and $(Na)_o$. The value of c can be estimated by fitting Eq. 10 with the data of V in K- and Na-free ASW. In Fig. 1, the broken line is a theoretical curve based on Eq. 10. The value of b is taken as 0.01 and the value of c is 0.053. It seems that the modified GHK equation (Eq. 10) fits the experimental data as satisfactorily as the original GHK equation.

Inasmuch as the value of c is relatively small, the difference between the modified GHK equation and the commonly used GHK equation is not significant when the external concentration of K is high. However, at low $[K]_o$, such as that near the physiological state, where $(K)_o/(K)_i \leq 0.1$, the c term in Eq. 10 cannot be ignored. This means that the effects of divalent ions and the unstirred layer are important to the resting potential at the physiological state. They become even more important when the axon is bathed in K-free artificial sea water.

Using the modified GHK equation, the resting potential measured at different Na concentrations can be fitted easily. Such a fitting is shown in Fig. 2, where the broken line is the theoretical curve of Eq. 10, with values of b and c identical to those used in fitting the curve in Fig. 1 (b = 0.01, c = 0.053). Thus, Eq. 10 can fit the V vs. $[Na]_o$ data satisfactorily. Comparison between the fittings of Eq. 2 and Eq. 10 in Fig. 2 suggests that in a constant field model, it is important to take into consideration currents carried by divalent ions and effects of the unstirred layer and the periaxonal space.

The parameter c in Eq. 10 contains contributions from currents of divalent ions and effects of the unstirred layer. It would be more informative if one could estimate the contribution of each of the effects separately. Using the data reported in Table V, one can estimate that c approaches 0.035 as $[Ca]_o$ approaches zero. Because c = 0.053 provides the best fit for the data in Fig. 2, one can conclude that about one-third of the normal value of c is contributed by the Ca^{++} current. Assuming that Mg^{++} is roughly one-half as effective as Ca^{++} (Frankenhauser and Hodgkin, 1957), and recalling that $[Mg]_o/[Ca]_o = 2.5$, the effects of the unstirred layer (i.e., the term $J_K P_K \gamma_K / P_{DK}$) are estimated to account for approximately one-fourth of the c value.

Finally, I would like to point out a limitation of this work. The modified constant field equation used here may not be unique in its ability to explain the concentration dependence of V. Other equations that have a semilogarithmic dependence of V on ionic concentrations also might fit the data. Theories different from the constant field approach have been reported in the literature (see, for example, Tasaki, 1968; Schwartz, 1971; Chang, 1977; and Ling, 1982), and some of them predict a semilogarithmic dependence of V on $[K]_o$ and $[Na]_o$. What can be safely concluded from this study is that if one uses a constant field approach to explain the resting potential, the effects of divalent ions and of the unstirred layer cannot be ignored.

CONCLUSION

I have measured the resting potential in squid axons at various $[Cl]_i$, $[Cl]_o$, $[K]_o$, $[Na]_o$, and $[Ca]_o$. The resting potential is not sensitive to $[Cl]_i$, $[Cl]_o$, or $[Na]_o$, but it is sensitive to $[K]_o$ and $[Ca]_o$. Using a fixed value for the parameter P_{Na}/P_K, the GHK equation can be fitted to the V vs. $[K]_o$ data but not to the V vs. $[Na]_o$ data. If one modified the GHK equation by including the contribution of currents carried by divalent ions and the effects of the

unstirred layer and the periaxonal space, the constant field equation could fit the V vs. $[Na]_o$ data and explain why the resting potential is finite at zero $[K]_o$, $[Na]_o$, and $[Cl]_i$.

APPENDIX

This section contains a derivation of the modified GHK equation that takes into consideration the effect of divalent ions (Ca and Mg) on the resting potential. The assumptions used in deriving the constant field equation (Hodgkin and Katz, 1949) are applied here, except that the net current of the anion (Cl) is considered to be very small and thus can be ignored.

Starting from the Nernst-Planck differential equations, Hodgkin and Katz (1949) showed that under the constant field assumption the potassium current across the membrane is

$$I_K = P_K \frac{F^2V}{RT} \frac{(K)_i e^{VF/RT} - (K)_o}{1 - e^{VF/RT}}.$$

(Here V is defined as the potential inside minus the potential outside.) Let us denote

$$X = e^{VF/RT} \tag{A1}$$

then

$$I_K = P_K \frac{F^2V}{RT} \frac{(K)_i X - (K)_o}{1 - X}. \tag{A2}$$

Similarly,

$$I_{Na} = P_{Na} \frac{F^2V}{RT} \frac{(Na)_i X - (Na)_o}{1 - X}. \tag{A3}$$

Currents carried by divalent ions can be calculated by the same procedure. The results are similar to those for monovalent ions, except that $F \rightarrow 2F$ (and hence $X \rightarrow X^2$), i.e.,

$$I_{Mg} = P_{Mg} \frac{4F^2V}{RT} \frac{(Mg)_i X^2 - (Mg)_o}{1 - X^2} \tag{A4}$$

and

$$I_{Ca} = P_{Ca} \frac{4F^2V}{RT} \frac{(Ca)_i X^2 - (Ca)_o}{1 - X^2}. \tag{A5}$$

Because the internal concentration of Ca in most biological systems is practically zero, $(Ca)_i$ may be ignored and Eq. A5 becomes

$$I_{Ca} = -P_{Ca} \frac{4F^2V}{RT} \frac{(Ca)_o}{1 - X^2}. \tag{A6}$$

The steady-state condition requires

$$I_K + I_{Na} + I_{Mg} + I_{Ca} = 0. \tag{A7}$$

Substituting Eqs. A2, A3, A4, and A6 into Eq. A7 and using $(1 - X^2) = (1 - X)(1 + X)$, I have

$$P_K[(K)_i X - (K)_o] + P_{Na}[(Na)_i X - (Na)_o] + 4P_{Mg} \frac{(Mg)_i X^2 - (Mg)_o}{1 + X} - 4P_{Ca} \frac{(Ca)_o}{1 + X} = 0. \tag{A8}$$

This equation can be rearranged to become

$$X^2 + (1 - R_1)X - (R_1 + R_2) = 0 \tag{A9}$$

where

$$R_1 = \frac{P_K(K)_o + P_{Na}(Na)_o + 4P_{Mg}(Mg)_i}{P_K(K)_i + P_{Na}(Na)_i + 4P_{Mg}(Mg)_i} \tag{A10}$$

$$R_2 = \frac{4P_{Ca}(Ca)_o + 4P_{Mg}[(Mg)_o - (Mg)_i]}{P_K(K)_i + P_{Na}(Na)_i + 4P_{Mg}(Mg)_i}. \tag{A11}$$

The solution of Eq. A9 in which X has a positive value is

$$X = \tfrac{1}{2}\{(R_1 - 1) + [(1 - R_1)^2 + 4(R_1 + R_2)]^{1/2}\} = \tfrac{1}{2}(R_1 - 1) + \tfrac{1}{2}[(1 + R_1)^2 + 4R_2]^{1/2}. \tag{A12}$$

For most biological systems, $(Ca)_o < (K)_i$, and the permeabilities of the divalent ions are believed to be much smaller than P_K in the resting state. Therefore, I consider only the case $R_2 << 1$. Then, the solution of X can be simplified by expanding the square root in power series and ignoring the higher order terms, i.e.,

$$[(1 + R_1)^2 + 4R_2]^{1/2} = (1 + R_1)\left[1 + \frac{4R_2}{(1 + R_1)^2}\right]^{1/2} \simeq (1 + R_1)\left[1 + \frac{2R_2}{(1 + R_1)^2}\right].$$

Substituting into Eq. A12, one has

$$X = R_1 + \frac{R_2}{1 + R_1}. \tag{A13}$$

Because $R_2 << 1$, the second term on the right-hand side is nonnegligible only when $R_1 << 1$. Then, it is a good approximation that

$$X \simeq R_1 + R_2. \tag{A14}$$

Substituting A1, A10, and A11 into Relation A14, I have

$$V = RT/F \cdot \ln\left[\frac{P_K(K)_o + P_{Na}(Na)_o + 4P_{Ca}(Ca)_o + 4P_{Mg}(Mg)_o}{P_K(K)_i + P_{Na}(Na)_i + 4P_{Mg}(Mg)_i}\right]. \tag{A15}$$

This work was supported by contract N0004-76-C-0100 from the Office of Naval Research.

I thank Professor R. Vick for careful reading of the manuscript, Steven Chan and Jeff Baron for technical assistance, and Ann Thompson and Gerrie Hazlewood for secretarial assistance.

Received for publication 24 November 1982 and in revised form 2 February 1983.

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