K Permeability of *Nitella clavata* in the Depolarized State

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**ABSTRACT**  Membrane current responses to sudden potential changes were recorded in solutions of various \([K]^+_o\) on 52 internodal cells of *Nitella clavata*. The membrane current after sudden depolarization had a component sensitive to \([K]^+_o\) which increased with time from 0.3 to 2.0 s and remained steady thereafter. This late current became zero at values of \(E\) and \([K]^+_o\), which suggests that the current was nearly all carried by \(K^+\). The potassium conductivity represented by this current increased with depolarization, with a half-maximum value at about \(-70\) mV, and saturation at about \(-30\) to \(-20\) mV. The potassium conductance also increased with increasing \([K]^+_o\), but less rapidly than predicted for constant potassium permeability. This failure of the conductance to increase with \([K]^+_o\) was relatively the same at all membrane potentials and may be explained by a model with a finite number of channels. No attempt was made to model the dependence of \(g_K\) on time after depolarization or on membrane potential. However, the finding that the membrane potential did not affect the way in which the permeability depended on \([K]^+_o\) suggests that the membrane potential change does not affect the affinity of the sites, and that the increase in \(g_K\) with time after depolarization is brought about by an increase in the number of channels with such sites.

**INTRODUCTION**

Since Hodgkin and Huxley (1952) successfully described the process of an action potential with the kinetics of the Na-conductance and K-conductance changes, the mechanism of the conductance change of excitable membrane has been investigated using many models (Mullins, 1959; Goldman, 1964; Hoyt, 1968; Tsien and Noble, 1969; and Moore and Jakobsson, 1971). In connection with the mechanism of control of ion movements across a membrane, these models may be classified into two groups: in the first, pathways specific for some ion species are blocked at their orifice by competing non-permeable ions, and the affinity of the sites at the orifices for the competing ions is a function of a membrane potential; in the second, the pathways for
specified ion species are blocked in the middle (or sometimes at the entrance) by molecules composing the pathway itself, and the conformation of the molecules would change as a function of membrane potential, while the affinity of the sites at the orifices of the pathways remains constant. In the first group, the gating particles are in the bathing solution, but in the second, they are within the membrane. We define a channel as a specific pathway which is not closed by molecules composing the pathway itself. In the first group an increase in permeability is not associated with an increase in the number of channels, while in the second an increase in permeability is a direct result of the increase in the number of channels.

A model belonging to the first group was adopted by Mullins (1959) to explain the dependence of the kinetics of Na-conductance change on the external Ca concentration. Similarly, Moore and Jakobsson (1971) further analyzed the process of the change in the Na conductance by using the relaxation method, and got a good fit with the experimental observation by Franken-haeuser and Hodgkin (1957). The original model used by Hodgkin and Huxley (1952) for the explanation of the transient increase in Na conductance and the late increase in K conductance on depolarization seems to belong to the second. The model explaining the late increase in K conductance in terms of a single channel by Mullins (1959) also belongs to the second. This kind of model was also adopted to account for the conductance changes in cardiac muscle by Noble and Tsien (1969).

Experiments reported here were aimed at estimating whether or not the increase in potassium permeability on depolarization is due to an increase in the affinity of sites at the orifice of the specific pathway or channel. The principle of the estimation was that when the number of channels for \( K^+ \) is limited, the dependence of the potassium permeability on \([K]_o\) at a constant membrane potential is determined by the affinity for \( K^+ \) of sites at the orifices of the channels.

**METHODS**

*Nitella clavata* internodal cells were used throughout the experiments. *Nitella* cells were cultivated in a solution similar to that reported by Barr (1965). After harvesting, internodal cells of about 2-mm length were kept in a normal artificial pond water (APW) of the following composition: 1.0 mM NaCl, 1.0 mM CaCl\(_2\), 1.0 mM MgSO\(_4\), and 0.05 mM K\(_2\)SO\(_4\). In order to measure the membrane current, an internodal cell was mounted in a chamber. The chamber was separated into three compartments by septa. General features of the chamber were almost identical with those previously reported (Kitasato, 1968), but the distance between the two septa which separate the central compartment from the two end compartments was 0.8 cm in order to reduce nonlinearity in the electrotonic potential of the part of the cell in the central compartment. The length of the cell in the central compartment was perfused extracellularly with a variety of test solutions, and the membrane potential of that part was controlled.
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by a voltage clamp circuit. Current responses to sudden potential changes were recorded at 3-min intervals. Membrane current measurements were done in 52 cells.

Test Solutions

High K test solutions were prepared by adding K$_2$SO$_4$ to the normal APW. The pH of the solutions was usually adjusted to 6.0 by adding H$_2$SO$_4$ except in the case in which the effect of pH changes was examined. High Cl test solutions were prepared by adding one of the following substances: NaCl, choline chloride, or Tris-HCl. High Ca test solutions were prepared by adding either CaCl$_2$ or Ca isethionate.

Measurement of the Internal K Concentration

Specially designed capillary-type glass electrodes (NAS 27-4)(Corning Glass Works, Corning, N. Y.) were used for measurements of the [K] in cell sap. The value of [K] of cell sap was estimated by inserting the measured value of the potential difference between the K-sensitive glass electrode and reference electrode into the graph which had been drawn by using the values of the potential difference in KCl solutions of various concentrations.

RESULTS

Effect of Cl-Concentration Change on the Transient Membrane Current. Before determining the magnitude of current carried by K$^+$ (K current or $I_K$) it may be necessary to know whether ion-carrying inward current during depolarization has any effect on the late outward current. Membrane current responses were recorded by using the voltage clamp at various [Cl]$^o$. A change in [Cl]$^o$ was made by adding NaCl, choline chloride, or Tris-HCl to normal APW. The [K]$^o$ in the test solutions was always 0.1 mM, and membrane potential was held at $-110$ mV. For convenience in comparing responses to the same depolarization in different solutions, the value of the command potential was fixed during the measurement of responses at different [Cl]$^o$, and then it was changed to a new value. Fig. 1 shows the records of the membrane current responses at [Cl]$^o$ of 3, 10, and 30 mM. The figures on the left side of the curves indicate the values to which the membrane potential was shifted from the holding potential. The general shape of the membrane current responses to sudden depolarization consisted of a transient inward current followed by a late steady outward current. As seen in Fig. 1, the transient inward current was divided into two parts. The initial inward current was not sensitive to the external Cl-concentration change, while the later part of the current was sensitive. The late steady outward current remained almost constant over the range of [Cl]$^o$ examined. The Cl-sensitive current began to appear at about 0.2 s from the beginning of the depolarization, reached the maximum value at about 0.6 s, and declined within 2 s.

Membrane current responses at 10 mM K were also recorded under conditions of various [Cl]$^o$. The difference between two responses at 1 mM Cl + 10
mM K APW and 10 mM Cl + 10 mM K APW was almost identical with that between responses at 1 mM Cl + 0.1 mM K APW and at 10 mM Cl + 0.1 mM K APW, even though at high [K]o the late current shifted downward. Thus, since [Cl]o change had no effect on the late current, and alternatively, the [K]o change did not influence the Cl-sensitive current which faded out 2 s after the beginning of the depolarization, it may be said that after 2 s there is substantially no Cl current in the membrane current.

Late Outward Current

The K activity in the cell sap was obtained by using a capillary-type electrode which was made from K-sensitive glass (Corning, NAS 27-4). The [K] of the cell sap used in the experiments shown in Fig. 2 corresponded to 75 mM KCl solution. Average value of the corresponding KCl concentration was 77 ± 3.2 mM. This value agrees well with that obtained by using K-sensitive glass microelectrodes on Nitella flexilis (Koltunov, 1963), and also with the values measured by flame photometry (MacRobbie, 1962; Spanswick and Williams, 1964). Since the activity coefficient of 77 mM KCl solution is 0.797 (MacInnes, 1961), the activity of K+ in cell sap was estimated as 61.3 mM. From this figure the values of $E_K$ at various [K]o were calculated as summarized in Table I.

The membrane current responses at various [K]o were recorded under voltage clamp conditions. The holding potential was −110 mV. The value of the command potential was changed after recording one series of responses to the same potential change at various [K]o. The curves a, b, c, d, and e in...
Table I

VALUE OF MEMBRANE POTENTIAL AT WHICH THE LATE CURRENT IS ZERO, AND \( E_K \) CALCULATED FROM THE K ACTIVITIES IN THE INTERNAL AND EXTERNAL SOLUTIONS

| \([K]_o\) | \((E)_{1=0}\) | \( E_K \) |
|---------|------------|-------|
| mM      | mV         | mV    |
| 0.1     | —          | -160  |
| 1.0     | —          | -102  |
| 3.0     | (-95)*     | -73   |
| 10.0    | -43 ± 2.1  | -44   |
| 30.0    | -21 ± 1.5  | -15   |

* The value was obtained by extrapolation.

Fig. 2 A were recorded at the concentrations of 0.1, 1, 3, 10, and 30 mM, respectively. The time-course of the delayed membrane current was independent of \([K]_o\), which can readily be seen by taking the difference between any two responses at different values of \([K]_o\).

The magnitudes of the late steady current were plotted against membrane potential \( E \) in Fig. 2 B. The values of \( E \) at which the late current becomes zero were consistent with the values of \( E_K \) calculated from the internal and external K activities (Table I). This finding suggests that the late steady current is nearly all carried by \( K^+ \).

Relation between the Chord K Conductance and the \([K]_o\).

The dependence of the K permeability on membrane potential may be represented qualitatively by the chord K conductance \( g_K \) defined as \( I_K/(E - E_K) \). In Fig. 3 the relation between \( g_K \) and the total membrane potential \( E \) at various \([K]_o\) is shown. As these curves show, the value of \( g_K \) was larger the greater the value of \([K]_o\), and depolarization also resulted in an increase in the value of \( g_K \), while the value saturated at around -30 to -20 mV.

In order to estimate the dependence of K permeability on \([K]_o\) from a comparison of magnitudes of \( I_K \) recorded at different \([K]_o\), one requires that the parameters contributing to the permeability, such as the number of channels, the mobility of \( K^+ \) within the channel, the length of the channel, and the affinity of site in the channel to ions, be unchanged even at different \([K]_o\). In the present experiments the addition of K to normal APW caused a considerable change in the ionic strength. This change in ionic strength is known to bring about a change in the surface potential and in the value of the transmembrane potential \( E_m \) even though the total membrane potential \( E \) is kept constant. It is widely accepted that the change in the surface potential would result in a shift of the dependence of \( g_K \) on \( E \) along the voltage axis (Chandler et al., 1965; Hille, 1968). However, as seen in Fig. 3, the \( g_K \) vs. \( E \) curves at 0.1, 1, 3, and 10 mM K were almost the same shape with a half-
Figure 2. A: Membrane current responses at various [K]o. Curves a, b, c, d, and e are the responses at 0.1, 1.0, 3.0, 10, and 30 mM K, respectively. Figures on the left side of curves indicate the values of the membrane potential to which the membrane potential was changed from the holding potential of -110 mV. The [K]o in a test solution was changed by adding K2SO4 to APW ([Ca]o = 1 mM, pH = 6). Scale for time is in seconds, and for current is in µA/cm². B: The relation between the magnitude of the late steady current and the membrane potential at various [K]o.

maximum value at about -70 mV and saturation at around -30 to -20 mV, while at 30 mM K the value of E at which gK was half-maximal shifted about 8 mV. These findings suggest that as long as the total membrane potential is the same, the parameters contributing to permeability remain unchanged over the range of [K]o from 0.1 to 10 mM.

Relation between the K Permeability and the [K]o

A net flux of ions across a membrane may be described by a product of a proportional coefficient (or apparent permeability coefficient) and the driving
force expressed in a form of electrochemical activity difference (Polissar, 1954). The apparent permeability coefficient should be distinguished from the permeability coefficient in the Goldman and Hodgkin-Katz formulation of ionic current (Goldman, 1943; Hodgkin and Katz, 1949). Denoting the apparent permeability coefficient for $K^+$ by $P_K$, the current carried by passive movement of $K^+$ across a membrane $I_K$ is described as follows:

$$I_K = FP_K([K]_i - [K]_o)e^{-\frac{\eta F}{RT}}$$

$$= FP_K[1 - e^{-\frac{(x-x_K)F}{RT}}]$$

where $F$, $R$, and $T$ have their usual meanings. $P_K$ may be related to the number of channels, to the mobility of $K^+$ in the channel, and to both gross partition coefficients at the inner and outer surfaces.

Eq. 1 \(b\) shows that if $P_K$ remains constant over the range of $[K]_o$ examined. The dependence of $I_K$ on $\exp \left[-(E - E_K)F/RT\right]$, in which $E_K$ is a function of $[K]_o$, is expressed by a straight line crossing the x axis at $x = 1$ (or $E - E_K = 0$). In Fig. 4 A and 4 B, the magnitude of the late steady currents is plotted against $\exp \left[-(E - E_K)F/RT\right]$ for membrane potentials of $-30$, $-50$, and $-60$ mV. Straight lines were drawn by assuming that the value of $P_K$ would remain at the level for $[K]_o = 0.1$ mM, even at higher $[K]_o$. Fig. 4 A is for the range of the positive value of $E - E_K$ (or $0 < x < 1$), and Fig. 4 B is for the range of the negative value of $E - E_K$ (or $1 < x$). The experimentally obtained values did not fit with the theoretical straight lines. This failure was approximately the same at all membrane potentials.
Figure 4. The relation between the late steady current and the driving force for K\(^+\) in a form of \(1 - \exp^{-r(E-E_K)F/RT}\). The straight lines show theoretical magnitudes of \(I_K\) when the apparent permeability coefficients remain in values of those at [K]\(_o\) = 0.1 mM. The figures on the right side of straight lines are the values of the membrane potential at which the currents were calculated. A is for the range of positive values of \(E - E_K\), B is for the range of negative values of \(E - E_K\). *, ●, □ and ○ represent the experimental magnitudes of the late steady currents at -30, -50, and -60 mV, respectively.

The ratio of the value of \(P_K\) at a given [K]\(_o\) to that at [K]\(_o\) = 0.1 mM \(P_K/(P_K)_{0.1}\) was graphically obtained by dividing the experimental value of the late steady current by the theoretical magnitude of \(I_K\) at the same membrane potential and [K]\(_o\). The values of \(P_K/(P_K)_{0.1}\) are summarized in Table II. These values were plotted against the logarithm of [K]\(_o\) without regard to membrane potentials. As shown in Fig. 5, experimental values of \(P_K/(P_K)_{0.1}\) decreased with an increase in [K]\(_o\) and were located around a single sigmoidal curve.

The solid curve in Fig. 5 is drawn by using a theoretical relation of a gross partition coefficient ratio to [K]\(_o\) obtained from the basic assumption that on the outer surface of a membrane the following reaction would occur.

\[
K + S \rightleftharpoons KS,
\]

where S is the free site at the outer orifice of the K channel and KS the site
TABLE II

APPROXIMATE PERMEABILITY COEFFICIENT RATIO AS A FUNCTION OF THE EXTERNAL K CONCENTRATION AT VARIOUS MEMBRANE POTENTIALS.

\begin{tabular}{|c|c|c|c|}
\hline
[K]o & mV & mV & mV \\
\hline
0.1 & 0.945 & 0.840 & 0.988 \\
1.0 & 0.745 & 0.740 & 0.795 \\
3.0 & 0.55 & 0.50 & 0.635 \\
10 & - & 0.291 & 0.240 \\
30 & - & - & - \\
\hline
\end{tabular}

bound with K+. \( K_1 \) is the dissociation constant of KS. If the site has some affinity to ions other than K+, a parallel reaction also should be considered. The parallel reaction is described in the following scheme,

\[ X + S \overset{K_s}{\longrightarrow} XS. \]

Since the number of sites for K+ at the outer surface is presumably finite, the maximum density of K+ in the membrane at the surface \((K)_\text{max}\) is equal to the sum of \([S]\), \([KS]\), and \([XS]\). By using this relation, the concentration of K+ in the membrane at the outer surface \((K)_o\) is obtained as follows:

\[ (K)_o = [KS] = \frac{[K]_o(K)_{\text{max}}Z_0^{-1}}{K_1(1 + [K]_oZ_0^{-1}/K_1 + [X]_oZ_0^{-n}/K_2)}, \] (2)

where \( Z_0^{-1} = \exp(-F\psi_o/RT) \), \( \psi_o \) is the outer surface potential defined with reference to the bulk of the external solution, and \( n \) is the valence of the competing ion X. Since the gross partition coefficient at the outer surface \( \beta_o \) is given by \((K)_o/[K]_o\), \( \beta_o \) is expressed by the following equation:

\[ \beta_o = \frac{(K)_{\text{max}}Z_0^{-1}}{K_1(1 + [K]_oZ_0^{-1}/K_1 + [X]_oZ_0^{-n}/K_2)}. \] (3)

The ratio of the gross partition coefficient at given \([K]_o\) to that at \([K]_o = 0\) is given as follows:

\[ \frac{\beta_o}{(\beta)_o} = \frac{K_1Z_0 + K_2[X]_oZ_0^{-n}/K_3Z_0^{-1}}{[K]_o + K_1Z_0 + K_2[X]_oZ_0^{-n}/K_3Z_0^{-1}}. \] (4)

where \((\beta)_o\) is the gross partition coefficient at \([K]_o = 0\). Eq. 4 shows that if \( K_1 \) and \( K_2 \) are not affected by a change in membrane potential, then for any value of membrane potential the relation of \( \beta_o/(\beta)_o \) to \log \([K]_o\) is expressed.
by a single sigmoidal curve, and the value of \([K]_a\) at \(\beta_s/\langle \beta_s \rangle_\infty = 1/2\) is equal to \(K_2Z_o(1 + [X]Z_o^{-1}/K_3)\).

As seen in Fig. 5, the experimental \(P_K/(P_K)_0\) vs. \([K]_0\) curve shows a good fit with a single theoretical \(\beta_s/\langle \beta_s \rangle_\infty\) vs. \([K]_a\) curve. This finding suggests that the permeability is proportional to the gross partition coefficient at the outer surface. Furthermore, the fact that the values of \(P_K/(P_K)_0\) were independent of membrane potential and depended solely on \([K]_a\) implies that the values of \(K_1\) and \(K_2\) are not affected by a change in membrane potential. It must be noted, however, that at \([K]_a = 30\) mM the agreement of the experimental values with the theoretical gross partition coefficient ratios was rather fortuitous, because at that concentration the surface potential change produced by

\[ \text{FIGURE 5. The relation between } P_K/(P_K)_0\text{ (ratio of the apparent K-permeability coefficient at a given } [K]_a\text{ to that at 0.1 mM K) and log}[K]_a\text{. } \circ, \square, \text{ and } \\circ \text{ represent experimental ratios at } -30, -50, \text{ and } -60 \text{ mV. The curve represents the theoretical dependence of } \beta_s/\langle \beta_s \rangle_\infty \text{ (the ratio of the gross partition coefficient at a given } [K]_a\text{ to that at } [K]_a = 0\text{) on } [K]_a. \]  

the change in \([K]_a\) was estimated not to be negligible as described before. This point will be discussed later.

**Effect of pH Change in the External Solution on the Late Steady Current**

Some attempts to estimate the affinity of the site to \(H^+\) were made by changing pH in the external solution. The membrane current responses at various pH are shown in Fig. 6. The test solution contained 1 mM K and 1 mM Ca. Experiments were performed mainly at pH greater than 6, because the responses at pH lower than 5 were not stable enough to be analyzed systematically. As these records show, the external pH change produced neither significant change in the magnitude of the late steady current nor a shift of the dependence of \(g_K\) on \(E\) along the voltage axis. These findings imply that the sites of K channels are almost completely in dissociated form over the
range of pH higher than 6, and the density of fixed charges on the outer surface is not influenced by the pH change.

**Effect of the [Ca] Change on the Late Steady Current**

In the presence of Ca++ in the external solution the resting membrane potential is not sensitive to a change in [K]s, and with a decrease in [Ca], it becomes sensitive to [K]s (Barr, 1965; Kitasato, 1968). This finding seems to imply that Ca++ may act as a competing ion at the outer orifice of the K channel. Membrane current responses to various potential changes were recorded at [Ca], of 1, 5, and 15 mM. Test solutions were prepared by adding either Ca isethionate or CaCl2 to 1 mM K APW. The membrane current responses at values of [Ca], less than 1 mM were not systematically examined, because the responses were not reproducible.

The magnitude of the late steady current decreased with an increase in the [Ca]s. The values of gK were plotted against membrane potential. As the curves in Fig. 7 show, with an increase in the [Ca], the gK vs. E curve shifted to the positive side. The few measured shifts of the value of E at which gK was half-maximum were 7 mV at 5 mM Ca and 14 mV at 15 mM Ca. Calculation from these values shows that a 10-fold increase in divalent ions (to 20 mM, because the normal APW contains 1 mM Ca and 1 mM Mg ions) produces a shift of the gK vs. E curve of about 16.5 mV in the range of [Ca]
from 1 to 10 mM. This finding suggests that the outer surface of the membrane bears fixed negative charges which are screened by Ca++. Besides the hyperpolarizing effect of Ca++ on $E_m$, the maximum value of $g_K$ also decreased to a small extent with an increase in $[Ca]$ from 1 to 15 mM. This finding implies that a small fraction of sites at the outer orifice K channels is blocked by Ca++ at the higher concentration, but not in such a manner that depolarization removes Ca++ from the sites. The increase in K permeability with a decrease in $[Ca]$ may be accounted for mainly by the increase in the outer surface potential, or by the depolarizing effect on $E_m$.

**DISCUSSION**

It is reasonable to consider that the number of channels is not unlimited. For example the density of Na channels in lobster nerve was estimated as 13 channels/μm² axon surface from the experiments with tetrodotoxin (TTX) (Moore et al., 1967). The similar situation may be considered on the K permeation of Nitella cells. Permeability change may be explained in two different ways, each with a limited number of channels. One of the ways is based on the idea that conformation of molecules composing the channel is a function of the membrane potential, and the other is based on the idea that impermeable ions (such as Ca++) block the channels, and this blockage is a function of membrane potential.

If the K permeability increase in a Nitella cell results from a channel conformation change, it might be expected that the increase in K permeability on depolarization is not directly related to an increase in the affinity of the K site for K⁺, since the increase in K permeability is caused solely by the increase in the number of K channels. On the contrary, if the K permeability increase is a consequence of the release of blocking ions from the sites at the orifices of K channels, the increase in the K permeability should be related to the increase in the apparent affinity for K⁺, or to the decrease in that for the blocking ions. The affinity of a site may be represented by dissociation con-
stants $K_1$ and $K_2$ of site-ion complexes. Eq. 4 shows that if $K_1$ and/or $K_2$ are functions of membrane potential, the $\beta_0/(\beta_0)\ast$ vs. log $[K]$ curve should shift with membrane potential change. On the contrary, if $K_1$ and $K_2$ are not functions of membrane potential, the $\beta_0/(\beta_0)\ast$ vs. log $[K]$ curve should remain in the same position independently of the value of the membrane potentials. The ratio of gross partition coefficients may be given experimentally by the ratio of the apparent permeability coefficients at different $[K]_o$, because even if the number of K channels is a function of membrane potential, at the same membrane potential the number of channels is considered the same, and the mobility of K$^+$ in a channel is not expected to be a function of the $[K]_o$.

In order to obtain the $P_K/(P_K)_{o,1}$ at various $[K]_o$, some problems about the outer surface of the membrane must be discussed. In the experiments with Nitella cells it is impossible to increase the $[K]$ in the test solution without changing the ionic strength of the solution; the concentration of ions in normal APW is too low to permit adjusting the ionic strength by subtracting other ions from normal APW. One might argue that the inevitable change in ionic strength will bring about outer surface potential change which prevents a change in ionic concentration just outside of the membrane proportional to the change in concentrations in the bulk solution. If there were a number of fixed charges at the surface of a membrane, it would be expected that a surface potential would be of considerable magnitude. For comparison of the experimental conditions it will be helpful to calculate the relative screening abilities of K$^+$ and Ca$^{++}$ or Mg$^{++}$. The fixed negative charge density on the inner surface of squid giant nerve fibers was estimated as $1.4 \times 10^{13}$ electronic charges/cm$^2$ which generated the inner surface potential of 38 mV at $[KCl]_i$ of 50 mM (Chandler et al., 1965). The outer surface potentials was estimated by Hille (1968) on the Ranvier node of myelinated nerve fibers as about $-70$ mV with a charge density of $3.5 \times 10^{13}$ electronic charges/cm$^2$. The charge density on the surface of a plasma membrane of Nitella has not been determined yet. However, the cell wall structure outside of the plasma membrane was reported to have cation exchange properties (Gaffey and Mullins, 1958), and this cell wall structure forms diffuse Donnan layer (Hope and Walker, 1961). From these findings it would be rational to assume that there is some difference between potentials just outside of the membrane and those in the bulk solution. Even though there was a considerable surface potential at the outer surface, the important point in this discussion is whether the magnitude of the boundary potential would change with a change in $[K]_o$ under the present experimental conditions. MacLaughlin et al., (1971) showed that the relative screening abilities of monovalent and divalent ions under the condition of $|\psi_o| > RT/F$ are represented by the following equation,

$$C^{++} = \frac{(C^+)^2}{(272 \sigma)^3}.$$
where \( \sigma \) is the charge density in \( \text{charges/} \AA^2 \). If the negative fixed charge density of the membrane of \textit{Nitella} were about the same order as that of nerve fiber, the magnitude of the outer surface potential would be calculated as about \(-80\text{mV}\) in normal APW. A divalent ion concentration of 2 mM can be calculated to produce the same screening effect as \([K]_o\) of 42 mM. These figures seem to account for the experimental findings that \( g_K \) vs. \( E \) curves were almost in the same position over the range of \([K]_o\) of 0.1–10 mM while at 30 mM K the curve shifted to an extent of 8 mV along the voltage axis (Fig. 3). Since the K concentrations used in the present experiments were in the range of 0.1 to 30 mM and the total concentration of divalent ions was 2 mM, it may be safely considered that the change in the \([K]_o\) of 0.1–10 mM produces little change in the outer surface potential. The surface potential change produced by increasing the \([K]_o\) to 30 mM may result in a less increase in \(["K"]_o\) just outside of the membrane to the extent of \( \text{exp} \left[-\Delta \psi, F/RT\right] \). This error may lead to an overestimation of the gross partition coefficient to the extent of 1.38. On the other hand, the hyperpolarizing effect of 30 mM K on \( E_m \) by about 8 mV may result in a decrease in \((K)_{max}\). The latter error brings about an underestimation of the gross partition coefficient. The extent of the underestimation is supposed to be about 0.75 from the dependence of \( g_K \) on \( E \) shown in Fig. 3. These errors may cancel with each other, and lead to agreement of the experimental \( P_K/(P_{K'})_{0.1} \) with the theoretical \( \beta_e/\beta_o \).

The present experiments show that the membrane potential does not affect the way in which K permeability depends on \([K]_o\), although K permeability depends on \( E \) most significantly. This finding suggests that the membrane potential does not affect the affinity of the sites, and that the increase in K permeability is brought about by an increase in the number of K channels with such sites.

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