# Ionic currents of channels that are permeable to monovalent and divalent cations

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ABSTRACT The cation-selective channel from *Tetrahymena* cilia is permeable to both monovalent and divalent cations. The single channel conductance in mixed solutions of $K^+$ and $Ca^{2+}$ was determined by the Gibbs-Donnan ratio of $K^+$ and $Ca^{2+}$, and the binding sites of this channel were considered to be always occupied by two potassium ions or by one calcium ion under the experimental conditions: 5–90 mM $K^+$ and 0.5–35 mM $Ca^{2+}$ (Oosawa and Kasai, 1988). A two-barrier model for the channel was introduced and the values of Michaelis-Menten constants and maximum currents carried by $K^+$ and $Ca^{2+}$ were calculated using this model. Single channel current amplitudes and reversal potentials were calculated from these values. The calculated single-channel currents were compared with those obtained experimentally. The calculated reversal potentials were compared with the resting potentials of *Tetrahymena* measured in various concentrations of extracellular $K^+$ and $Ca^{2+}$. The method of calculation of ionic currents and reversal potentials presented here is helpful for understanding the properties of the channels permeable to both monovalent and divalent cations.

## INTRODUCTION

Many channels are known to be permeable not only to divalent cations but also to monovalent cations. These include calcium channels (Kostyuk et al., 1983; Almers and McCleskey, 1984; Hess and Tsien 1984; Hess et al., 1986; Coronado and Affolter, 1986) and the nicotinic acetylcholine receptor channel (Dani and Eisenman, 1987). Hess and Tsien (1984) have discussed the mechanism of ion permeation through calcium channels. They pointed out that the channel is occupied almost continually by one or more calcium ions under physiological conditions.

It is important to construct an ion permeation model for channels that are permeable to both divalent and monovalent cations (such as $Ca^{2+}$ and $K^+$). We showed previously that the single channel conductance of the *Tetrahymena* cation channel was controlled by the Gibbs-Donnan ration and proposed a new model for the permeation of monovalent and divalent cations through this channel (Oosawa and Kasai, 1988). Here I improve this model by using a two-barrier model to calculate the single channel current and the reversal potential of the channel from the concentrations of monovalent and divalent cations on each side of the membrane. The derived values of the channel currents and the reversal potentials of the channel are helpful for an understanding of the channel properties.

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## MODEL FOR ION PERMEATION

One of the simplest models for ion permeation through a channel is a one-ion channel model (see Läuger, 1973; Hille, 1975, 1984). This model assumes that (*a*) ions must bind to certain sites in the pore as part of the permeation process, and (*b*) the channel can contain only one ion at a time in the pore. These assumptions are analogous to those made in enzyme kinetics. For an understanding of a two-ion channel model it is first necessary to consider a model in which the channel can contain only a single ion.

### $K^+$ solution

#### One-ion channel model

First, I shall consider that the channel has one binding site (B) with an energy barrier on each side and that the permeating cation is potassium ($K^+$). A representation of this two-barrier model is shown in Fig. 1. The permeation step can be described as follows:

$$K_o^+ + B \underset{k_{K-1}}{\overset{k_{K1}}{\rightleftharpoons}} BK^+ \underset{k_{K-2}}{\overset{k_{K2}}{\rightleftharpoons}} B + K_i^+, \qquad (1)$$

where the rate constants are dependent on voltage. The net current is the difference between the influx and efflux. From chemical kinetics, the steady-state current in the outward direction can be expressed (Hille, 1984) as

$$I_K = \frac{I_{K,i,max}[K]_i/K_{K,i} - I_{K,o,max}[K]_o/K_{K,o}}{1 + [K]_i/K_{K,i} + [K]_o/K_{K,o}}, \qquad (2)$$

where $I_K$ represents the total potassium current, and


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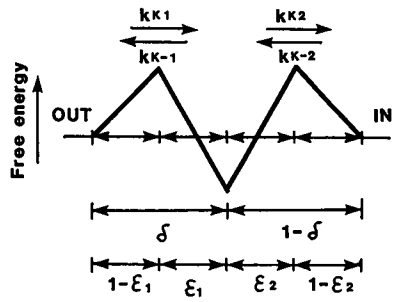


FIGURE 1 The two-barrier model. A hypothetical channel is shown with two barriers and one binding site. The rate constants $k_{K1}$ and $k_{K-2}$ are rates of binding, whereas $k_{K-1}$ and $k_{K2}$ are rates of release. The energy profile for the hypothetical channel is shown with the energy well located at $\delta$. The quantities $\epsilon_1$ and $\epsilon_2$ represent the fractions of the electrical potential drop between the site and the energy maxima.

$I_{K,o,max}$ and $I_{K,i,max}$ represent the maximum current for $K^+$ moving from the outside to the inside of the membrane and from the inside to the outside, respectively. The Michaelis-Menten constants ($K_{K,i}$, $K_{K,o}$) can be expressed in terms of rate constants.

$$K_{K,0} = (k_{K-1} + k_{K2})/k_{K1} \quad (3)$$

$$K_{K,i} = (k_{K-1} + k_{K2})/k_{K-2}, \quad (4)$$

where $K_{K,o}$ and $K_{K,i}$ represent the Michaelis-Menten constants for the outside ion and the inside ion, respectively. These constants are voltage dependent. The rate constants are voltage dependent and can be written as

$$k_{K1} = k_{Kb} \exp [F\delta(-V)(1 - \epsilon_1)/RT] \quad (5)$$

$$k_{K-2} = k_{-Kb} \exp [F(1 - \delta)V(1 - \epsilon_2)/RT] \quad (6)$$

$$k_{K2} = k_{Kf} \exp [F(1 - \delta)(-V)\epsilon_2/RT] \quad (7)$$

$$k_{K-1} = k_{-Kf} \exp (F\delta V\epsilon_1/RT), \quad (8)$$

where $F$, $R$, and $T$ are the usual thermodynamic quantities. The quantity $\delta$ represents the fraction of the total electrical potential drop, $V$, between the outside of the membrane voltage field and the ion binding site (Oosawa and Sokabe, 1986), where 0 corresponds to the outside and 1 to the inside of the membrane. It is often called the electrical distance of the site from the outside of the membrane. The quantities $\epsilon_1$ and $\epsilon_2$ represent the fractions of the electrical potential drop between the ion binding site and the energy maxima (Fig. 1). $k_{Kf}$, $k_{-Kf}$, $k_{Kb}$, and $k_{-Kb}$ represent the voltage-independent elements of the rate constants.

I assume that the two energy maxima are equal and the barriers symmetrical so that the value of $\delta$ is 0.5, and $\epsilon_1$ equals $\epsilon_2(=\epsilon)$, because experimentally the single-channel conductance was the same, whether the net current was inward or outward (Oosawa and Sokabe, 1985). At first, the value of $\epsilon$ was set at 0.5. The rate constant $k_{Kf}$ equals $k_{-Kf}$ and $k_{Kb}$ equals $k_{-Kb}$, because the values of free energy between the binding site and the two energy maxima were considered to be the same. When the ion concentrations outside and inside are the same, Eq. 2 becomes

$$I_K = \frac{(I_{K,i,max}/K_{K,i} - I_{K,o,max}/K_{K,o})[K]}{1 + [K]/[K_{K,i}K_{K,o}/(K_{K,i} + K_{K,o})]}. \quad (9)$$

Eq. 9 can be reduced to the familiar Michaelis-Menten equation.

$$I_K = \frac{(I_{K,max,m}/K_{K,m})[K]}{1 + [K]/K_{K,m}}, \quad (10)$$

with

$$K_{K,m} = K_{K,i}K_{K,o}/(K_{K,i} + K_{K,o}) \quad (11)$$

$$I_{K,max,m} = \frac{I_{K,i,max}/K_{K,i} - I_{K,o,max}/K_{K,o}}{1/K_{k,o} + 1/K_{k,i}}, \quad (12)$$

where $K_{K,m}$ represents the Michaelis-Menten constant and $I_{K,max,m}$ represents the maximum single channel current.

## Two-ion channel model in which each ion permeates independently

I shall now extend the single-ion channel model to a two-ion channel model in which the pore can contain two ions at the same time. We have already shown that the cation channel from *Tetrahymena* ciliary membrane can contain two potassium ions in the pore simultaneously (Oosawa and Kasai, 1988). I postulate that each potassium ion permeates through the channel independently, because in $K^+$ solution the channel showed a Michaelis-Menten type saturation curve (Oosawa et al., 1988). In this case, the equation for the net current becomes Eq. 2, that is, the two-ion model also shows a Michaelis-Menten type saturation curve when the ion concentrations are the same on both sides.

Michaelis-Menten constants and maximum currents are voltage dependent. $K_{K,o}$ and $K_{K,i}$ can be expressed in terms of the Michaelis-Menten constant at $V = 0$. From Eqs. 3, 5, 7, and 8,

$$K_{K,o} = \frac{K_K\{\exp [F(1 - \delta)(-V)\epsilon/RT] + \exp (F\delta V\epsilon/RT)\}}{2\exp (F\delta(-V)(1 - \epsilon)/RT)} \quad (13)$$

From Eqs. 4 and 6–8

$$K_{K,i} = \frac{K_K\{\exp [F(1 - \delta)(-V)\epsilon/RT] - \exp (F\delta V\epsilon/RT)\}}{2\exp (F(1 - \delta)V(1 - \epsilon)/RT)}, \quad (14)$$

where $K_K$ represents the Michaelis-Menten constant at

$V = 0$. $I_{K,o,max}$ and $I_{K,i,max}$ can be expressed in terms of the maximum current for $K^+$ at $V = 0$.

$$I_{K,o,max} = I_{K,max} \exp [F(1 - \delta)(-V)\epsilon/RT] \quad (15)$$

$$I_{K,i,max} = I_{K,max} \exp (F\delta V\epsilon/RT) \quad (16)$$

$$I_{K,max} = 2Fk_{Kf}, \quad (17)$$

where $I_{K,max}$ represents the maximum current at $V = 0$. If the two-ion channel shows a Michaelis-Menten type saturation curve, $K_K$ and $I_{K,max}$ can be calculated from the Michaelis-Menten constant and the maximum current.

The values of the Michaelis-Menten constant and maximum current of this *Tetrahymena* cation channel at +25 mV were 16.3 mM and 8.85 pA, respectively (Oosawa et al., 1988). From these values, I obtained the values for $K_K$ and $I_{K,max}$ of 32.6 mM and 17.5 pA, respectively. Fig. 2 shows the I-V relations for the K current in symmetrical 20 mM K solutions and with 20 mM K externally and 100 mM internally calculated using these values. The cation-selective channel from *Tetrahymena* ciliary membrane is permeable to both $K^+$ and $Ca^{2+}$ ions. When both $K^+$ and $Ca^{2+}$ are present in the solution, $Ca^{2+}$ blocks the movement of $K^+$ because it binds more strongly to the ion binding site within the pore. In mixed cation solutions the channel thus shows both saturation and competition.

## Mixed solution of $K^+$ and $Ca^{2+}$

When $K^+$ and $Ca^{2+}$ are both present, we get not only saturation but also competition between ions. The cation channel from *Tetrahymena* ciliary membrane can contain two potassium ions or one calcium ion in the pore simultaneously (Oosawa and Kasai, 1988). The total current ($I_t$) of this cation channel with ions on both sides becomes

$$I_t = I_{K,i} + I_{Ca,i} - I_{K,o} - I_{Ca,o} \quad (18)$$

$$I_{K,o} = \frac{I_{K,o,max}([K]_o^2/K_{K,o}^2 + [K]_o/K_{K,o} + [K]_o[K]_i/K_{K,o}K_{K,i})}{A} \quad (19)$$

$$A = 1 + 2[K]_o/K_{K,o} + [K]_o^2/K_{K,o}^2 + 2[K]_i/K_{K,i} + [K]_i^2/K_{K,i}^2 + 2[K]_o[K]_i/K_{K,o}K_{K,i} + [Ca]_o/K_{Ca,o} + [Ca]_i/K_{Ca,i} \quad (20)$$

$$I_{K,i} = \frac{I_{K,i,max}([K]_i^2/K_{K,i}^2 + [K]_i/K_{K,i} + [K]_o[K]_i/K_{K,o}K_{K,i})}{A} \quad (21)$$

$$I_{Ca,o} = \frac{I_{Ca,o,max}[Ca]_o/K_{Ca,o}}{A} \quad (22)$$

$$I_{Ca,i} = \frac{I_{Ca,i,max}[Ca]_i/K_{Ca,i}}{A}. \quad (23)$$

$I_{K,o}$ and $1_{K,i}$ represent the current for $K^+$ from outside to inside (influx) and from inside to outside (efflux), respectively. The Michaelis-Menten constants ($K_{Ca,o}$ and $K_{Ca,i}$) can be expressed in terms of rate constants and they can be calculated from the Michaelis-Menten constant for $Ca^{2+}$ at $V = 0$ ($K_{Ca}$).

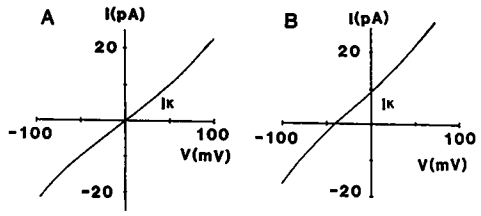


FIGURE 2 I-V relation of the two-ion channel with 20 mM $K^+$ on both sides ($A$), and with 100 mM $K^+$ inside and 20 mM $K^+$ outside ($B$). $\delta = 0.5$. $\epsilon_1 = \epsilon_2 = 0.5$.

$$K_{Ca,o} = \frac{K_{Ca}\{\exp [2F(1 - \delta)(-V)\epsilon/RT] + \exp (2F\delta V\epsilon/RT)\}}{2 \exp (2F\delta(-V)(1 - \epsilon)/RT)} \quad (24)$$

$$K_{Ca,i} = \frac{K_{Ca}\{\exp [2F(1 - \delta)(-V)\epsilon/RT] + \exp (2F\delta V\epsilon/RT)\}}{2 \exp (2F(1 - \delta)V(1 - \epsilon)/RT)}. \quad (25)$$

Rate constants are given as follows.

$$k_{Ca1} = k_{Cab} \exp [2F\delta(-V)(1 - \epsilon)/RT] \quad (26)$$

$$k_{Ca-2} = k_{-Cab} \exp [2F(1 - \delta)V(1 - \epsilon)/RT] \quad (27)$$

$$k_{Ca2} = k_{Caf} \exp [2F(1 - \delta)(-V)\epsilon/RT] \quad (28)$$

$$k_{Ca-1} = k_{-Caf} \exp (2F\delta V\epsilon/RT), \quad (29)$$

where $k_{Caf}$, $k_{-Caf}$, $k_{Cab}$, and $k_{-Cab}$ represent the rate constant of $Ca^{2+}$. The rate constant $k_{Caf}$ equals $k_{-Caf}$ and $k_{Cab}$ equals $k_{-Cab}$. At $V = 0$, $k_{Ca1}$ and $k_{Ca-2}$ equal $k_{Caf}$ so that the differences of free energy between the binding site and the two energy maxima at $V = 0$ are the same.

$I_{Ca,o,max}$ and $I_{Ca,i,max}$ can be expressed in terms of the maximum current for $Ca^{2+}$ at $V = 0$ ($I_{Ca,max}$).

$$I_{Ca,o,max} = I_{Ca,max} \exp [2F(1 - \delta)(-V)\epsilon/RT] \quad (30)$$

$$I_{Ca,i,max} = I_{Ca,max} \exp (2F\delta V\epsilon/RT) \quad (31)$$

$$I_{Ca,max} = 2Fk_{Caf}. \quad (32)$$

The probabilities that the channel is empty [$P$(B)], or contains one outside $K^+$ [$P$(BKo)], one inside $K^+$ [$P$(BKi)], two outside $K^+$ [$P$(BKo2)], two inside $K^+$ [$P$[BKi2)], one outside $K^+$ and one inside $K^+$ [$P$(BKio)], one inside $Ca^{2+}$ [$P$(BCai)], or one outside $Ca^{2+}$ [$P$(BCao)] are given as follows.

$$P(B) = 1/A \quad (33)$$

$$P(\mathrm{BKo}) = \frac{2[\mathrm{K}]_o/\mathrm{K}_{\mathrm{K},o}}{A} \quad (34)$$

$$P(\mathrm{BKi}) = \frac{2[\mathrm{K}]_i/\mathrm{K}_{\mathrm{K},i}}{A} \quad (35)$$

$$P(\mathrm{BKo2}) = \frac{[\mathrm{K}]_o^2/\mathrm{K}_{\mathrm{K},o}^2}{A} \quad (36)$$

$$P(\mathrm{BKi2}) = \frac{[\mathrm{K}]_i^2/\mathrm{K}_{\mathrm{K},i}^2}{A} \quad (37)$$

$$P(\mathrm{BKio}) = \frac{2[\mathrm{K}]_o[\mathrm{K}]_i/\mathrm{K}_{\mathrm{K},o}\mathrm{K}_{\mathrm{K},i}}{A} \quad (38)$$

$$P(\mathrm{BCao}) = \frac{[\mathrm{Ca}]_o/\mathrm{K}_{\mathrm{Ca},o}}{A} \quad (39)$$

$$P(\mathrm{BCai}) = \frac{[\mathrm{Ca}]_i/\mathrm{K}_{\mathrm{Ca},i}}{A}, \quad (40)$$

where

$$P(B) + P(\mathrm{BKo}) + P(\mathrm{BKi}) + P(\mathrm{BKo2}) + P(\mathrm{BKi2}) + P(\mathrm{BKio}) + P(\mathrm{BCao}) + P(\mathrm{BCai}) = 1. \quad (41)$$

These probabilities can be calculated from the ion concentrations and applied voltages.

## Saturation of the sites by two potassium ions or one calcium ion

When $K^+$ and $Ca^{2+}$ were present at high concentrations, the channel always contained two potassium ions or one calcium ion experimentally (Oosawa and Kasai, 1988). In this condition and when the ion concentrations are the same on both sides, each current becomes

$$I_{\mathrm{K},o} = \frac{(I_{\mathrm{K},o,\max}/\mathrm{K}_{\mathrm{K},o}^2 + I_{\mathrm{K},o,\max}/\mathrm{K}_{\mathrm{K},o}\mathrm{K}_{\mathrm{K},i})[\mathrm{K}]^2/[\mathrm{Ca}]}{B} \quad (42)$$

$$B = (1/\mathrm{K}_{\mathrm{K},o}^2 + 1/\mathrm{K}_{\mathrm{K},i}^2 + 1/\mathrm{K}_{\mathrm{K},o}\mathrm{K}_{\mathrm{K},i})[\mathrm{K}]^2/[\mathrm{Ca}] + 1/\mathrm{K}_{\mathrm{Ca},o} + 1/\mathrm{K}_{\mathrm{Ca},i} \quad (43)$$

$$I_{\mathrm{K},i} = \frac{(I_{\mathrm{K},i,\max}/\mathrm{K}_{\mathrm{K},i}^2 + I_{\mathrm{K},i,\max}/\mathrm{K}_{\mathrm{K},o}\mathrm{K}_{\mathrm{K},i})[\mathrm{K}]^2/[\mathrm{Ca}]}{B} \quad (44)$$

$$I_{\mathrm{Ca},o} = \frac{I_{\mathrm{Ca},o,\max}/\mathrm{K}_{\mathrm{Ca},o}}{B} \quad (45)$$

$$I_{\mathrm{Ca},i} = \frac{I_{\mathrm{Ca},i,\max}/\mathrm{K}_{\mathrm{Ca},i}}{B}. \quad (46)$$

The total current is determined by $[K]^2/[Ca]$, that is, by the Gibbs-Donnan ratio ($[K^+]/\sqrt{[Ca^{2+}]}$). As we reported before (Oosawa and Kasai, 1988), in a mixed solution of $K^+$ and $Ca^{2+}$ the values of single channel current were similar at similar values of the Gibbs-Donnan ratio. By setting the values of $K_K$ and $I_{K,max}$ to be 32.6 mM and 17.5 pA, respectively, net current was calculated from Eqs. 42–46 using the values of the Gibbs-Donnan ratio (Fig. 3). From curve fitting in Fig. 3, I obtained the values of $I_{Ca,max}$ and $K_{Ca}$ as 0.651 pA and 0.195 mM, respectively.

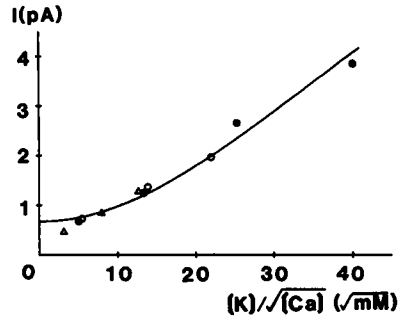


FIGURE 3 Single-channel currents determined by the Gibbs-Donnan ratio ($[K^+]/\sqrt{[Ca^{2+}]}$). The data in Table 1 (Oosawa and Kasai, 1988) were used. Applied voltage was +25 mV. $[K^+] + 2[Ca^{2+}] = 100$ mM (●), 30 mM (○), 10 mM (△).

If the value of $\epsilon$ is not 0.5, the values of $I_{K,max}$, $K_K$, $I_{Ca,max}$, and $K_{Ca}$ changed. I calculated these values for various values of $\epsilon$ (Table 1). The smaller the value of $\epsilon$, the larger the values of $I_{K,max}$, $K_K$, $I_{Ca,max}$, and $K_{Ca}$. As we mentioned before (Oosawa et al., 1988; Oosawa and Kasai, 1988), this channel was highly permeable to $K^+$, while it had a high-affinity $Ca^{2+}$-binding site.

If the values of $I_{K,max}$, $K_K$, $I_{Ca,max}$, and $K_{Ca}$ can be obtained, the values of ionic currents and the reversal potentials can be calculated from the ionic concentrations of $K^+$ and $Ca^{2+}$ on each side.

## Resting potentials of *Tetrahymena*

The resting membrane potential of *Tetrahymena* was depolarized by the addition of external $K^+$ ions and external $Ca^{2+}$ ions (Onimaru et al., 1980; Connolly and Kerkut, 1981). This suggests there may be channels through which $K^+$ and $Ca^{2+}$ can permeate which are open

**TABLE 1 Michaelis-Menton constants and maximum currents at $V = 0$**

| $\epsilon$ | 0.1 | 0.3 | 0.5 | 0.7 | 0.9 |
|---|---|---|---|---|---|
| $K_K$ ($mM$) | 35.9 | 34.2 | 32.6 | 31.0 | 29.6 |
| $I_{K,max}$ ($pA$) | 18.7 | 18.0 | 17.5 | 17.2 | 17.0 |
| $K_{Ca}$ ($mM$) | 0.284 | 0.236 | 0.195 | 0.161 | 0.136 |
| $I_{Ca,max}$ ($pA$) | 0.820 | 0.722 | 0.651 | 0.606 | 0.583 |

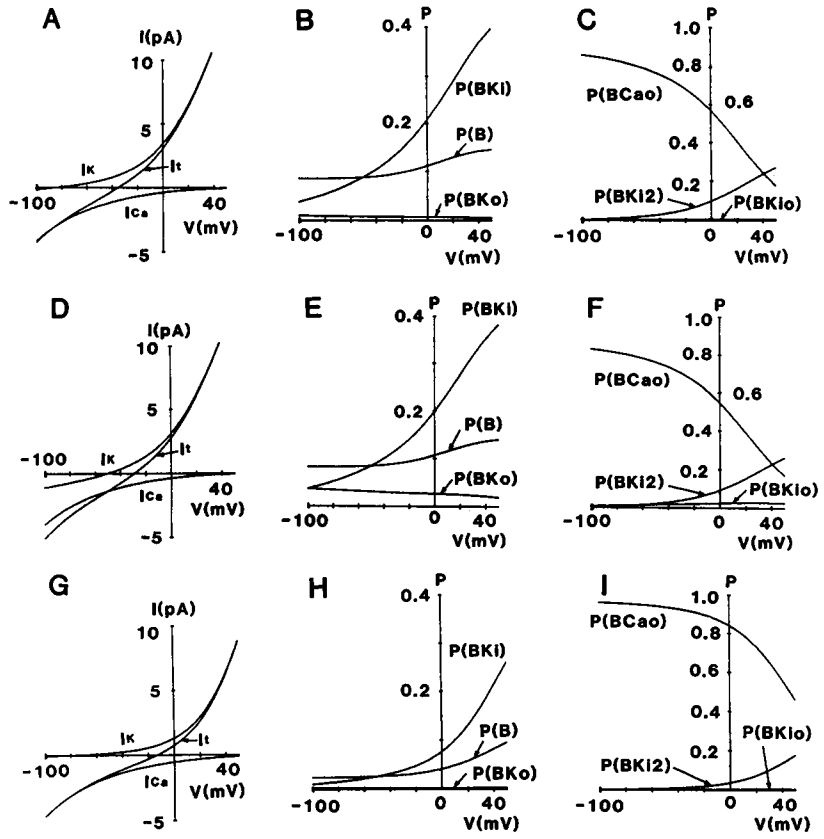


FIGURE 4 I-V relation of calculated ionic current and probability of ion occupancy in an asymmetric solution of $K^+$ and $Ca^{2+}$. $[K^+]_i$ = 30 mM, $[Ca^{2+}]_i$ = 0.1 $\mu$M, $\epsilon$ = 0.5. $P$(BKo2) and $P$(BCai) were very small, overlapping the abscissa here. (*A–C*) $[K^+]_o$ = 1 mM, $[Ca^{2+}]_o$ = 1 mM. (*D–F*) $[K^+]_o$ = 4 mM, $[Ca^{2+}]_o$ = 1 mM. (*G–I*] $[K^+]_o$ = 1 mM, $[Ca^{2+}]_o$ = 4 mM.

at the resting potential in the membrane of *Tetrahymena*. We have already reported that a cation channel that was incorporated into a planar lipid bilayer may contribute to the resting potential of *Tetrahymena* (Oosawa and Sokabe, 1985; Oosawa et al., 1988). To examine this possibility further, I used a two-ion channel model to calculate the single channel current amplitudes and the probabilities of ion occupancy of the channel (Fig. 4). In these calculations I assumed the intracellular $K^+$ concentration to be 30 mM (Dunham and Child, 1961), and the intracellular $Ca^{2+}$ concentration to be 0.1 $\mu$M (the intracellular concentration of free $Ca^{2+}$ appears to be <1 $\mu$M in *Paramecium* (Naitoh and Kaneko, 1972)). First I calculated currents when the extracellular $K^+$ concentration and the extracellular $Ca^{2+}$ were 1 mM. Then I changed either the external $K^+$ concentration, or the external $Ca^{2+}$ concentration, to 4 mM. In both cases the reversal potentials became more positive.

I compared the resting potentials measured experimentally with the reversal potentials calculated from Eqs. 18–23 (Fig. 5). In an external solution containing 1 mM KCl and 1 mM $CaCl_2$, the resting membrane potential of *Tetrahymena* was about −30 mV (Onimaru et al., 1980). The calculated reversal potential in this solution was −35.2 mV when the value of $\epsilon$ was 0.5. There was thus some difference between the measured resting potential and the calculated reversal potential. Fig. 5 *A* illustrates the effect of external $[K^+]$ in both these parameters. As the value of $\epsilon$ was reduced, the calculated reversal potential became less negative. For example, when the value of $\epsilon$ is 0.9, the calculated reversal potential is −38.8 mV, whereas when the value of $\epsilon$ is 0.1, the calculated reversal potential becomes −32.9 mV. However, the reversal potential is not very sensitive to changes in the value of $\epsilon$ except at K concentrations between 1 mM and 2 mM (Fig. 5 *A*).

The membrane was depolarized by increasing external $Ca^{2+}$ by ~20 mV per 10-fold increase in the $Ca^{2+}$ concentration (Onimaru et al., 1980). However, as shown in Fig. 5 *B* the calculated reversal potentials increased more steeply with external $Ca^{2+}$ ions; for example by ~36 mV per 10-fold increase in the $Ca^{2+}$ concentration when

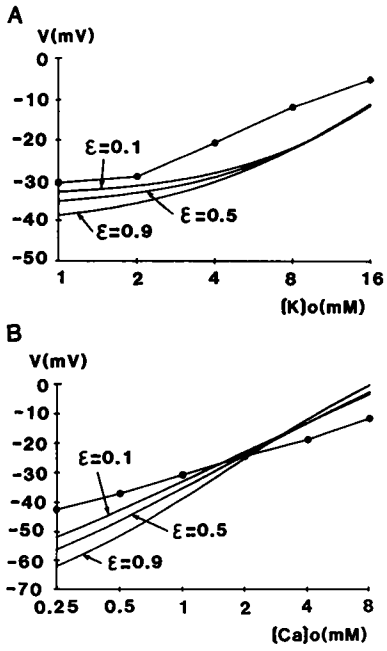


FIGURE 5 Concentration dependence of resting potentials and reversal potentials in mixtures of $K^+$ and $Ca^{2+}$. The symbols show the measured resting potentials of *Tetrahymena* (data from Onimaru et al., 1980). The continuous lines are calculated reversal potentials. (*A*) External $Ca^{2+}$ was held constant at 1 mM throughout while the external $K^+$ concentration was varied. (*B*) External $K^+$ was held constant at 1 mM throughout, whereas the external $Ca^{2+}$ concentration was varied.

$\epsilon = 0.5$. Again the calculated reversal potentials did not agree with the resting potentials of *Tetrahymena* (Fig. 5 *B*).

## DISCUSSION

In earlier experiments with mixed solutions of $K^+$ and $Ca^{2+}$ the channel currents were correlated with the Gibbs-Donnan ratio ($[K^+]/\sqrt{[Ca^{2+}]}$). It was therefore postulated that the channel could contain up to two $K^+$ or one $Ca^{2+}$ ions in experimental solutions containing 5–90 mM $K^+$ and 0.5–35 mM $Ca^{2+}$, and the channel was saturated and contained either two $K^+$ or one $Ca^{2+}$ ions. The value of $K_k$ was 32.6 mM, and $K_{Ca}$ was 0.2 mM. These results indicate that $Ca^{2+}$ ions bind considerably more tightly to the binding site within the pore than $K^+$ ions.

In this paper I show that it is possible to describe the resting potential in various ionic solutions by a two-ion channel model of a cation-selective channel permeable to both $K^+$ and $Ca^{2+}$. This channel has been reported in *Tetrahymena* previously. My interpretation is then different from previous models which assume that the resting potential is given by separate channels permeable either to $K^+$ or to $Ca^{2+}$ (Naitoh and Eckert, 1974).

The calculated reversal potential was −35.2 mV, whereas the resting potential of *Tetrahymena* was about −30 mV when external $K^+$ and $Ca^{2+}$ were 1 mM. The calculated reversal potentials were always less than the resting potentials of *Tetrahymena*.

When the external K and Ca were 1 mM, the calculated reversal potential was −35.2 mV close to the resting potentials of −30 mV. Furthermore the relationship between $[K]_o$ and reversal potential (in the presence of 1 mM Ca) was similar to that found for the resting potential. This suggests that the cation-selective channel contributes a large part of the resting conductance of *Tetrahymena* membrane. However the resting potential was always slightly less negative than the calculated reversal potentials under the same conditions. This raises the possibility that there may be other channels (less K-selective) which also contribute to the membrane potential. This idea is further supported by my finding that the calculated reversal potential changes more steeply with $[Ca]_o$ than does the resting potential. One possible explanation for this may be that other channels impermeable to divalent cations contribute to the resting membrane conductance.

The method of calculation of ionic currents and reversal potentials presented here should be helpful for understanding the properties of the channels that are permeable to monovalent and divalent cations.

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