Biochimica et Biophysica Acta, 692 (1982) 89–96
Elsevier Biomedical Press

BBA 71377

INHIBITION OF A VOLTAGE-DEPENDENT CATION CHANNEL IN SARCOPLASMIC RETICULUM VESICLES BY CAESIUM STUDIED BY USING A POTENTIAL-SENSITIVE CYANINE DYE

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(Received April 19th, 1982)

Key words: Cation channel; Cs^+ blockade; Cyanine dye; (Sarcoplasmic reticulum)

The effect of caesium on the cation transport system in sarcoplasmic reticulum vesicles has been analysed kinetically through $Tris^+$ influx. The $Tris^+$ influx was measured by following the change in K^+ diffusion potential due to the mutual diffusion between K^+ and $Tris^+$ in the presence of valiomycin using a potential probe; 3,3'-dipropylthiadicarbocyanine iodide. The main results were as follows. (1) $Tris^+$ influx increased when membrane potential became inside-negative. This suggests that $Tris^+$ permeates through the channel which has a voltage-dependent gate. (2) Cs^+ reacted with the cation transport system only from the outside of the vesicle and inhibited $Tris^+$ influx. The inhibition follows a single-site titration curve with a voltage-dependent dissociation constant of 18 mM at −60 mV. The inhibition can be explained by assuming that Cs^+ binds to a site located about 45% of the way through the membrane from the outside of the vesicle in the open state of the channel. These results are in good agreement with those reported by Coronado and Miller (Coronado, R. and Miller, C. (1979) Nature 288, 495–497), which were gained electrically by using sarcoplasmic reticulum vesicles incorporated into an artificial planar phospholipid bilayer.

Introduction

Miller and his collaborators [1–3] demonstrated by an electrical method that there exists a voltage-gated cation channel in sarcoplasmic reticulum vesicles which were incorporated into an artificial phospholipid bilayer. Of these works, that by Coronade and Miller [2] demonstrated that Cs^+ blocked the K^+ conductance of this channel. The blockade followed a single-site titration curve with a voltage-dependent dissociation constant of 18 mM at −50 mV. In the previous paper [4], we characterized the passive cation transport in intact sarcoplasmic reticulum vesicles by measuring choline influx in terms of a light-scattering method [5] and found that the nature of choline transport in the vesicles was very similar to that of the K^+ conductance reported by Miller and co-workers [1–3] with respect to for example, pH and temperature dependence. In particular, $choline^+$ influx was also inhibited by Cs^+. Thus, we suggest that a quaternary ammonium ion such as $choline^+$ also permeates through the same cation channel as K^+. However, we have little biochemical information about the voltage-dependent cation channel in intact sarcoplasmic reticulum vesicles, since the electrical method can measure properties only of those vesicles which can be incorporated into the artificial bilayer.

The present work is an attempt to elucidate the relationship between the cation transport in sarcoplasmic reticulum vesicle and membrane potential in vitro by using a fluorescent cyanine dye. The fluorescent cyanine dye, 3,3'-dipro-

Abbreviation: Mes, 2-(*N*-morpholino)ethanesulfonic acid.

pylthiadicarbocyanine iodide, is a well characterized potential probe which is able to follow cation flux by measuring membrane potential [6,7]. By this method, we performed a kinetic analysis of the reversible inhibition of the net cation flux by Cs^+. For this purpose, however, a standard monovalent cation, K^+, is unsuitable because its permeation is too fast to be measurd by the usual tracer method [1,5,8,9]. We employed a quaternary ammonium ion, $Tris^+$, because the permeability for $Tris^+$ is as small as choline and its movement can be measured by the light-scattering, potential-probe and tracer methods [5,9]. The $Tris^+$ influx was measured by following the change in K^+ diffusion potential. The K^+ diffusion potential was formed by diluting the vesicles incubated in a potassium-gluconate medium into a Tris-gluconate medium, and was monitored by using the cyanine dye. Since the $Tris^+$ influx is rate-limiting in the mutual diffusion process between K^+ and $Tris^+$, we could evaluate the rate constant of $Tris^+$ influx in addition to the magnitude of change in membrane potential.

As a result, we found that both $Tris^+$ influx and its Cs^+ blockade were voltage-dependent. These data suggest that $Tris^+$ permeates through the same voltage-gated cation channel as K^+. Thus, we could study the properties of the cation channel by the biochemical technique.

Materials and Methods

Preparation

Sarcoplasmic reticulum vesicles were isolated from rabbit dorsal and hind leg muscle as a microsomal fraction by the method of Weber et al. [10] with slight modification [11]. The final pellet was suspended in 20 mM potassium-Mes (pH 6.8), washed once, and stored at 0°C. The prepared vesicles were used within 1 week after isolation. The protein concentration of the vesicles in this suspension was usually 15–20 mg protein/ml. The protein concentration was estimated by means of the biuret reaction using bovine serum albumin as a standard.

Reagents

Potassium-gluconate and Tris-gluconate were made up as 1 M stock solutions, treated with activated charcoal, and filtered. The fluorescent cyanine dye, 3,3′-dipropylthiadicarbocyanine iodide (NK-2241), was purchased from Japanese Research Institute for Photosensitizing Dyes Co., Ltd, Japan. [^{14}C]Choline was purchased from CEA, France. Other reagents were commercial products of analytical grade.

Fluorescence measurements

Measurements of the fluorescence intensity of the cyanine dye to monitor the K^+-diffusion potential in sarcoplasmic reticulum vesicles were carried out by the methods described earlier [6,7]. The fluorescence intensity was measurd with a 1 cm square cell by using a fluorescence spectrophotometer (Union FS-501, Japan). The excitation and emission slits were set to give half-bandwidths of 7 nm. Fluorescence emission was recorded at 670 nm with excitation at 620 nm. Temperature was maintained at 23°C with a temperature controlling apparatus (Lauda-K2R, F.R.G.).

Determination of the rate constant of $Tris^+$ influx.

A typical experiment for measurement of $Tris^+$ influx was as follows. Sarcoplasmic reticulum vesicles were preincubated in 10 mM Tris-Mes (pH 6.8)/200 mM potassium-gluconate/5 mg protein/ml at 0°C overnight. A 20 μl aliquot of the suspension was diluted 100-fold into 2 ml of a medium containing 200 mM Tris-gluconate/10 mM Tris-Mes (pH 6.8)/2 μM cyanine dye/1 μM valinomycin and change in fluorescence was recorded. A rapid decrease in fluorescence (not recorded) took place due to the formation of an inside-negative potential in the vesicles caused by the K^+ gradient, and recovery of the fluorescence then followed (see curve A in Fig. 1). The later decrease in K^+ diffusion potential is due to a decrease in the intravesicular K^+ concentration, as the extravesicular K^+ concentration is constant. The intravesicular K^+ concentration may decrease through the mutual diffusion between K^+ and $Tris^+$ and through the co-diffusion with $gluconate^-$. However, since the fluorescence change in Fig. 1A was not affected when an inhibitor of the gluconate transport was applied [7], we neglected the effect of anion transport in the following analysis. Thus, since the permeability for $Tris^+$ is much smaller than that for K^+, $Tris^+$ influx is

rate-limiting and the following equation must hold;

$$-\frac{d[K]_i}{dt} = \frac{d[Tris]_i}{dt} = k_{Tris}[Tris]_o \quad (1)$$

where $[K]_i$, $[Tris]_i$, and $[Tris]_o$ are the concentrations of intravesicular K^+ and intravesicular and extravesicular $Tris^+$, respectively, and k_{Tris} is the rate constant of $Tris^+$ influx. Since membrane potential (E_m) is given by

$$E_m = -\frac{RT}{F}\ln\frac{[K]_i}{[K]_o} \quad (2)$$

and the extravesicular K^+ concentration ($[K]_o$) is constant, the following equation is obtained:

$$\frac{dE_m}{dt} = -\frac{RT}{F}\frac{1}{[K]_i}\frac{d[K]_i}{dt} \quad (3)$$

where F, R and T and Faraday's constant, the gas constant and absolute temperature, respectively. Now, the change in the fluorescence intensity ($\Delta F/F$) is proportional to that of the membrane potential; the rate constant of the fluorescence change (k_F) is thus given by:

$$k_F = \frac{d/dt(\Delta F/F)_{t=0}}{(\Delta F/F)_{t=0}} = \frac{(dE_m/dt)_{t=0}}{(E_m)_{t=0}} \quad (4)$$

where the subscript $t = 0$ shows the values at zero time. From Eqns. 1–4, the initial rate constant of $Tris^+$ influx can be calculated from the initial slope of the fluorescence change according to:

$$k_{Tris} = k_F\frac{\ln([K]_{i,t=0}/[K]_o)}{[Tris]_o/[K]_{i,t=0}} \quad (5)$$

The magnitude of change in membrane potential can be calculated by the percentage change in the fluorescence intensity corresponds to a 1.4 mV change in membrane potential [6] unless otherwise stated.

Measurement of the efflux rate of choline

The efflux rate of choline was measured by the Millipore filtration technique as described before [4].

Results

Effect of Cs^+ on $Tris^+$ influx

Fig. 1 shows a typical result of experiments designed to investigate the effect of Cs^+ on the permeation rate of $Tris^+$ influx. After incubation in 10 mM Tris-Mes (pH 6.8) and 200 mM potassium gluconate, sarcoplasmic reticulum vesicles were diluted 100-fold into a medium comprising 10 mM Tris-Mes (pH 6.8)/200 mM Tris-gluconate/2 μM cyanine dye/1 μM valinomycin, and the change in fluorescence intensity was followed (curve A). According to previous papers [6,7], the initial rapid decrease in fluorescence (not recorded) is due to the formation of an inside-negative membrane potential in the vesicles and the later recovery in fluorescence is due to a decrease in membrane potential as a result of the mutual diffusion between K^+ and $Tris^+$. As described in 'Materials and Methods', the initial rate constant of $Tris^+$ influx can be calculated from the initial slope of the fluorescence change using Eqn. 5. k_F and k_{Tris} were about $1.95 \cdot 10^{-2}$ s^{-1} and $8.54 \cdot 10^{-2}$ s^{-1}, respectively. This corresponds to a $Tris^+$ influx of 89.7 nmol/mg per s at 23°C on the as-

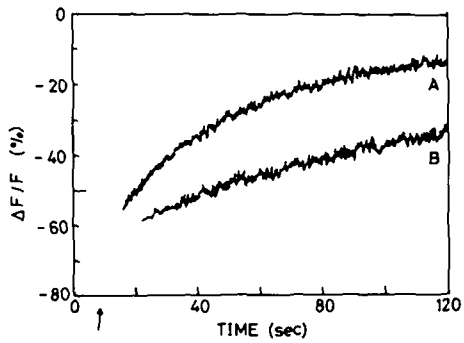

Fig. 1. Time course of fluorescence change in a voltage-sensitive cyanine dye caused by change in the K^+ diffusion potential. A 20 μl aliquot of a suspension of sarcoplasmic reticulum vesicles (5.0 mg protein/ml) incubated with 10 mM Tris-Mes (pH 6.8)/200 mM potassium gluconate was diluted 100-fold into 2 ml of a medium comprising 2 μM 3,3′-dipropylthiadicarbocyanine iodide/10 mM Tris-Mes (pH 6.8)/1 μM valinomycin/200 mM Tris-gluconate in the absence (A) or presence (B) of 10 mM Cs^+. The temperature was kept at 23°C. The ordinate shows the percentage change in fluorescence intensity. Cs^+ was added at the time indicated by the arrow ($t = 8$ s).

sumption that the intravesicular water space of the vesicles is 5 μl/mg [12,13].

When Cs^+ (10 mM) was added to the dilution medium, the influx of $Tris^+$ was slowed significantly (curve B). It is clear that Cs^+ did not affect the magnitude of the initially formed membrane potential when $Tris^+$ influx was reduced by more than 50% (compare A and B in Fig. 1). Because the permeability for K^+ is much higher than that for $Tris^+$ or Cs^+ in the presence of valinomycin, the reduction of the $Tris^+$ permeation by Cs^+ did not affect the K^+ diffusion potential.

In order to obtain the information about the Cs^+ permeability, the change in membrane potential was followed when the vesicles incubated in a 200 mM Cs_2SO_4 medium were diluted 100-fold into 200 mM $(Tris)_2SO_4$. Because no fluorescence change was observed under this condition, the permeability for Cs^+ must not be higher than that for $Tris^+$ (data not shown). Although extravesicular Cs^+ inhibited the influx of $Tris^+$, intravesicular Cs^+ upto 50 mM did not affect the influx of $Tris^+$ (data not shown). Thus, the reaction site for Cs^+ of the cation transport system was suggested to be located outside the sarcoplasmic reticulum vesicle. These results are in agreement with those obtained from the measurement of K^+ conductance by Coronado and Miller [2].

In order to investigate the effect of cyanine dye on the permeability for $Tris^+$, experiments similar to those shown in Fig. 1 were carried out with alteration in dye concentration. As a result, it was found that the cyanine dye in the present experiment did not affect $Tris^+$ permeability.

Dose-response of the inhibition of $Tris^+$ influx by Cs^+

In order to study the effect of extravesicular Cs^+ on $Tris^+$ influx quantitatively, experiments similar to those in Fig. 1 were carried out at different Cs^+ concentrations. The magnitude of membrane potential formed at the beginning was maintained at -110 mV, calculated by Eqn. 2. As shown in Fig. 2, the inhibition of $Tris^+$ influx by Cs^+ follows a single-site titration curve. The half-maximum inhibition of $Tris^+$ influx occurred at about 4.8 mM (apparent inhibition constant). However, the maximum inhibition did not reach 100%, but rather, 80%. This result suggests that

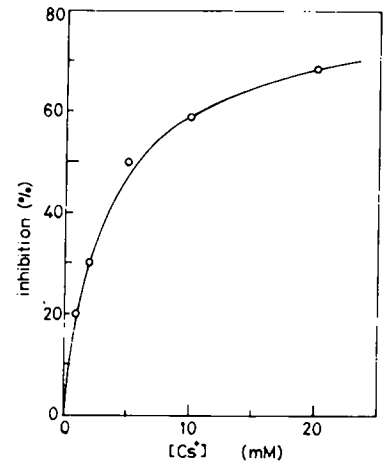

Fig. 2. Inhibition of $Tris^+$ influx as a function of Cs^+ concentration. Experments similar to those in Fig. 1 were carried out by changing the Cs^+ concentration. $Tris^+$ influx was measured as described in 'Materials and Methods'. Fractional inhibition (i) was defined as $(1-k/k_0)$, where k and k_0 are the initial rates of $Tris^+$ influx measured with and without Cs^+, respectively. The initial value of membrane potential in the vesicles was -110 mV from Eqn. 2.

two types of vesicle exist in our preparation: one type (80%) has channels which can be blocked by Cs^+ and the other (20%) has no such channel. This idea is also supported by the results of McKinley and Meissner [9].

Voltage-dependent Cs^+ inhibition of choline efflux

In order to confirm the above result by other methods, the efflux rate of $choline^+$, which is an organic cation similar to $Tris^+$, was measured by using radioactive tracer under conditions similar to those described in Fig. 1 in the absence and presence of membrane potential. The reason for our using choline instead of $Tris^+$ is that a radioactive tracer of choline was easily available. As shown in Fig. 3A, the choline efflux was little affected by 10 mM Cs^+ in the absence of membrane potential. On the contrary, the choline efflux was inhibited by 50% in the presence of an inside-negative membrane potential of -118 mV (Fig. 3B). The magnitude of the inhibition of $choline^+$ efflux by Cs^+ was similar to that of $Tris^+$ influx measured by fluorescence technique (compare Fig. 1 and Fig. 3B). These results show that the fluorescence method, by using a

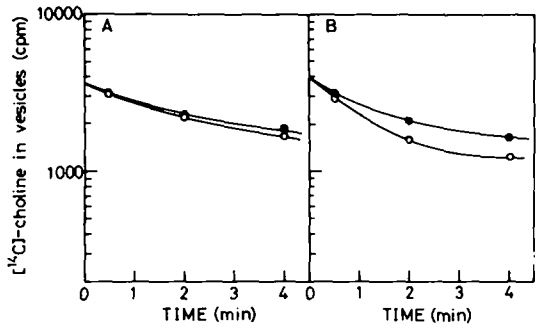

Fig. 3. *Time course of choline efflux and its voltage-dependent inhibition by Cs⁺.* Choline efflux was measured by using radioactive tracer under conditions similar to Fig. 1. A 40 μl aliquot of the vesicles incubated in 10 mM Tris-Mes (pH 6.8), 10 mg protein/ml, 1 mM choline chloride + [^{14}C]choline chloride and 200 mM potassium gluconate was diluted 100-fold into 4 ml of the dilution medium containing 10 mM Tris-Mes (pH 6.8), 1 mM choline Cl and either 200 mM potassium gluconate (A) or 200 mM Tris-gluconate (B) in the absence (○) and presence (●) of 10 mM Cs^+. Membrane potentials were calculated to be 0 mV in A and −118 mV in B.

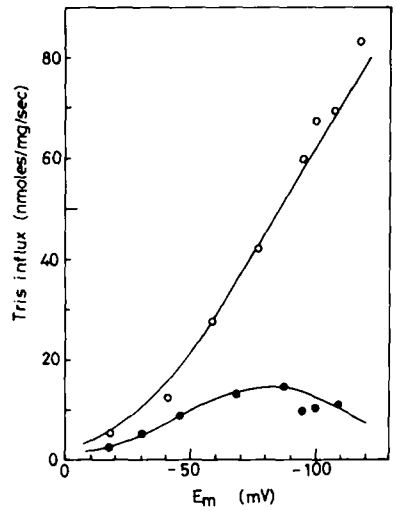

Fig. 4. Voltage dependence of $Tris^+$ influx and the effect of Cs^+. *Experiments similar to those in Fig. 1 were carried out.* The vesicles incubated in 10 mM Tris-Mes (pH 6.8), 5 mg protein/ml and 200 mM potassium gluconate were diluted 100-fold into 200 mM mixture of potassium gluconate and Tris-gluconate to form various magnitudes of membrane potential indicated on the abscissa in the absence (○) and presence (●) of 50 mM Cs^+. The magnitude of membrane potential in the absence of Cs^+ was calculated from Eqn. 2 and that in the presence of Cs^+ was calculated from the fluorescence intensity when Cs^+ was added by using the relationship that a 1% change in fluorescence intensity corresponds to a 1.4 mV change in membrane potential [6]. *The upper solid line is* drawn according to Eqn. 10 described in 'Discussion', with $L(0)=0.2$, $z=-1.0$ and $P_{Tris}[Tris]_o=16.8$ nmol/mg per s.

potential-sensitive dye, is a useful tool to measure the $Tris^+$ influx and to analyse the effect of Cs^+ on it.

Voltage dependence of $Tris^+$ influx and the effect of Cs^+

In the previous section, it was shown that the inhibition of choline$^+$ efflux by Cs^+ is voltage-dependent. In this section, the voltage dependence of $Tris^+$ influx and the effect of Cs^+ are examined in detail by using fluorescent cyanine dye. Membrane potentials of various magnitudes were formed by changing the concentrations of both K^+ and $Tris^+$ in the dilution medium. The results are shown in Fig. 4. The $Tris^+$ influx increased when membrane potential became inside-negative. For example, $Tris^+$ influx increased from 5.45 nmol/mg per s to 84.2 nmol/mg per s when the membrane potential was polarized toward inside-negative from −18 mV to −118 mV. The work of McKinley and Meissner on $Tris^+$ influx [9] was thus confirmed. This voltage dependence of $Tris^+$ influx can partly be explained by the following effect. Because $Tris^+$ is a monovalent cation, the influx of $Tris^+$ is assumed to be influenced by electric field (membrane potential) according to the Goldman-Hodgkin-Katz equation [14,15]:

$$J_{Tris} = -P_{Tris}[Tris]_o \frac{FE/RT}{1-\exp(FE/RT)} \qquad (6)$$

where P_{Tris} is the permeability coefficient for $Tris^+$. However, the magnitude of the increase in $Tris^+$ influx by polarization was far larger than that expected from this equation. Eqn. 6 predicts that the influx of $Tris^+$ will increase 3.4-times with polarization from −18 mV to −118 mV. It is probable that the cation transport system mediating $Tris^+$ influx has a voltage-dependent gating mechanism. The gating mechanism may work such a way that equilibrium between the closed and open states shifts toward the open state when membrane potential polarizes to inside-negative (see 'Discussion').

The effects of Cs^+ on $Tris^+$ influx at various

magnitudes of membrane potential are also shown in Fig. 4. While the inhibition of $Tris^+$ influx was only 42% at −41 mV in 50 mM Cs^+, it reached 83% at −118 mV and the same Cs^+ concentration. These results strongly indicate that the Cs^+ inhibition is voltage-dependent.

Effect of membrane potential on the Cs^+ inhibition of $Tris^+$ influx

As indicated in the previous section, the Cs^+ inhibition was voltage-dependent. In order to analyse this in detail, experiments similar to those in Fig. 2 were carried out at various magnitudes of membrane potential. As shown in Fig. 5, double-reciprocal plots of fractional inhibition against the concentration of Cs^+ were linear. The slope of the lines represents the apparent inhibition constant of Cs^+ (the apparent K_i). The apparent K_i increased with increasing the membrane potential (depolarized). For example, K_i increased from 4.8 mM to 62.5 mM when membrane potential was depolarized from −110 mV to −31 mV.

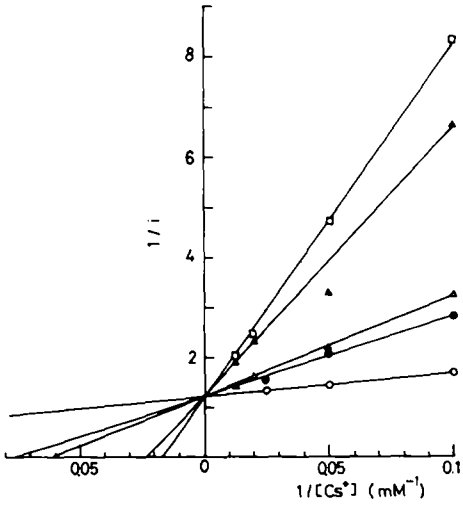

Fig. 5. Double-reciprocal plot of fractional inhibition vs. Cs^+ concentration. The data were taken from experiments similar to those in Fig. 2. The lines were drawn by eye. Various magnitudes of membrane potential were formed by changing the concentration of extravesicular K^+ as described in the legend to Fig. 4. The magnitude of membrane potential (mV) were as follows: ○, −110; ●, −86; △, −59; ▲, −46; □, −31.

Discussion

In this paper, we have found that $Tris^+$ influx in sarcoplasmic reticulum vesicles is voltage-dependent. When membrane potential became inside-negative from −18 mV to −118 mV, $Tris^+$ influx increased more than 10-fold. Furthermore, we found Cs^+ strongly blocked $Tris^+$ influx and its inhibition was voltage-dependent. Thus, it is reasonable to assume that $Tris^+$ permeates through the cation channel and its permeation is also controlled by the voltage-dependent gating process as reported by Labarca et al. [16]. Labarca et al. demonstrated that the cation channel has only two states, 'closed' and 'open', and the equilibrium between the closed and open states is voltage-dependent. The gating process was described by the conformational transition scheme:

$$\text{closed} \underset{L(E)}{\rightleftarrows} \text{open} \tag{7}$$

where $L(E)$ is the voltage-dependent equilibrium constant for channel opening. $L(E)$ varied exponentially with voltage according to:

$$L(E) = L(0)\exp(zFE/RT) \tag{8}$$

where $L(0)$ is the equilibrium constant at 0 mV and z is the parameter of the effective gating charge. Labarca et al. [16] showed that the value of $L(0)$ ranged between 0.14 and 0.23 (calculated from the values of internal free energy at pH 7.0) and the value of z was about −1.1. The voltage-dependent probability of any channel existing in the open state, $p(E)$, can be described as:

$$p(E) = \frac{L(0)\exp(zFE/TR)}{1 + L(0)\exp(zFE/RT)} \tag{9}$$

We would like to apply this model to the present results. According to the above model, $Tris^+$ influx mediated by the channel can be described as the following equation from Eqns. 6 and 9;

$$J_{Tris} = -P_{Tris}[Tris]_o \frac{(FE/RT)}{1-\exp(FE/RT)} \cdot p(E) \tag{10}$$

The upper solid line shown in Fig. 4 is drawn according to Eqn. 10 with $L(0) = 0.2$, $z = -1.0$, and $P_{Tris}[Tris]_o = 16.8$ nmol/mg per s. Thus, it is

plausible that $Tris^+$ permeates through the cation channel and the premeation is regulated by the voltage-dependent gating process.

In order to compare the inhibition of $Tris^+$ influx by Cs^+ with Cs^+ blockade of the macroscopic K^+ conductance [2], the apparent Cs^+ inhibition constant is plotted as a function of membrane potential formed in the vesicle membrane as shown in Fig. 6. Coronado and Miller [2] showed that the Cs^+ blockades of both microscopic and macroscopic K^+ conductance were voltage-dependent. The voltage-dependence was explained by assuming that Cs^+ bound to a site located about 40% of the way through the membrane from the *cis* side (the side to which the vesicles were added) in the open state of channel. According to Coronado and Miller [2], the kinetic scheme can be expressed by:

$$\text{closed} \underset{L(E)}{\rightleftarrows} \text{open} \overset{[Cs^+]}{\underset{K_b(E)}{\rightleftarrows}} \text{blocked} \tag{11}$$

where $K_b(E)$ is the voltage-dependent dissociation constant for Cs^+ binding, and 'blocked' is a state of the channel which has no conductivity due to the Cs^+ binding. Here, $K_b(E)$ varies exponentially with the voltage according to:

$$K_b(E) = K_b(0) \exp(\delta FE/RT) \tag{12}$$

where $K_b(0)$ is the zero-voltage dissociation constant, δ is the fraction of the total electrical potential drop across the membrane found at the Cs^+ binding site. Coronado and Miller [2] estimated $K_b(0)$ and δ to be 40 mM and 0.38, respectively. According to this model, the apparent inhibition constant of $Tris^+$ influx by Cs^+ can also be described by:

$$K_i = K_b(0) \exp(\delta FE/RT)/p(E) \tag{13}$$

The parameters obtained from the plot in Fig. 6 are $\delta = 0.45$ and $K_b(0) = 40$ mM. These values are in good agreement with those obtained by Coronado and Miller [2]. Therefore, these results strongly indicate that $Tris^+$ influx was inhibited due to the binding of Cs^+ to the channel. Further, these results support the idea that the *cis* side is the outside of the vesicles [1–3].

As far as the absolute value of the permeability for $Tris^+$ is concerned, it was calculated to be $1.4 \cdot 10^{-7}$ cm/s at -110 mV from Fig. 1 in the absence of Cs^+ under the assumption of the radius of sarcoplasmic reticulum vesicles to be 50 nm. This value is 2 orders larger than that obtained from the osmotic volume change measured by the light-scattering method [5,17]. One reason for this discrepancy is attributable to the difference in the experimental method. In the previous papers [5,17], an average value of the permeability over the different size of vesicle was obtained, while in the present paper, the permeability of the highly permeable vesicles was obtained. Another reason is the contribution of the membrane potential. The above figure is the maximal value obtained when most of the channels are open. In this paper, however, further analysis has not yet been done.

In the present study, we followed the behavior of the slowly permeable cations such as $Tris^+$. This is the reason we could detect the same cation channel as that reported by Coronado and Miller [2]. When we followed the permeation of K^+ by the light-scattering method [5], we found that Cs^+ did not inhibit the K^+ permeation and Cs^+ also permeated as quickly as K^+. This discrepancy may be attributable to the fact that K^+ permeates so fast that the time course of the fast component could not be followed even by the light-scattering method [5,18] and to the fact that K^+ and Cs^+ might permeate through the channel other than the voltage-gated one, such as Ca^{2+}-gated cation channel or Ca^{2+} channel [19,20].

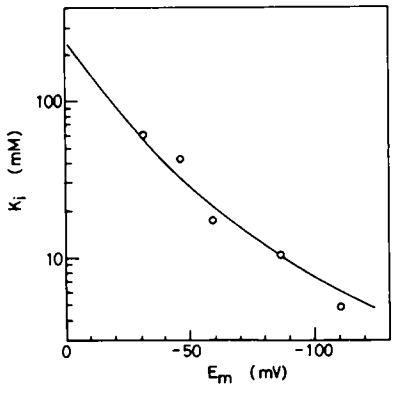

Fig. 6. Voltage dependence of the apparent inhibition constant of Cs^+, K_i. The data were taken from Fig. 5. The solid line is the experimentally fitted curve and is drawn according to Eqn. 13, with $K_b(0) = 40$ mM, $\delta = 0.45$, $L(0) = 0.2$ and $z = -1.0$.

Miller and his colleagues demonstrated the existence of the voltage-gated cation channel in sarcoplasmic reticulum vesicles [1–3,16]. Thus, sarcoplasmic reticulum membrane is in a sense electrically excitable. In this paper, we have reported a study on the voltage-gated cation channel in vitro by using a potential-sensitive fluorescent dye. This method must be a useful tool for studying various electrically excitable membranes in vitro.

Acknowledgements

Part of this work was supported by Grant-in-Aid for Scientific Research (5644085) from the Ministry of Education, Science and Culture of Japan.

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