ABSTRACT

Using the patch-clamp whole-cell recording technique, we investigated the influence of external Ca$^{2+}$, Ba$^{2+}$, K$^+$, Rb$^+$, and internal Ca$^{2+}$ on the rate of K$^+$ channel inactivation in the human T lymphocyte-derived cell line, Jurkat E6-1. Raising external Ca$^{2+}$ or Ba$^{2+}$, or reducing external K$^+$, accelerated the rate of the K$^+$ current decay during a depolarizing voltage pulse. External Ba$^{2+}$ also produced a use-dependent block of the K$^+$ channels by entering the open channel and becoming trapped inside. Raising internal Ca$^{2+}$ accelerated inactivation at lower concentrations than external Ca$^{2+}$, but increasing the Ca$^{2+}$ buffering with BAPTA did not affect inactivation. Raising [K$^+$]$_o$, or adding Rb$^+$ slowed inactivation by competing with divalent ions. External Rb$^+$ also produced a use-dependent removal of block of K$^+$ channels loaded with Ba$^{2+}$ or Ca$^{2+}$. From the removal of this block we found that under normal conditions ~25% of the channels were loaded with Ca$^{2+}$, whereas under conditions with 10 μM internal Ca$^{2+}$ the proportion of channels loaded with Ca$^{2+}$ increased to ~50%. Removing all the divalent cations from the external and internal solution resulted in the induction of a non-selective, voltage-independent conductance. We conclude that Ca$^{2+}$ ions from the outside or the inside can bind to a site at the K$^+$ channel and thereby block the channel or accelerate inactivation.

INTRODUCTION

Voltage-dependent K$^+$ channels in human T lymphocytes inactivate almost completely over a period of hundreds of milliseconds during depolarizing voltage steps (DeCoursey et al., 1984; Matteson and Deutsch, 1984). The inactivation rate is sensitive to the presence of divalent ions in the bathing solution; elevated external Ca$^{2+}$ or added lanthanum increase, whereas manganese and cobalt decrease, the rate of inactivation (DeCoursey et al., 1985). One possible explanation for these results is that Ca$^{2+}$ or lanthanum can gain access to a site that inactivates the channel, whereas manganese and cobalt may prevent Ca$^{2+}$ from gaining access to this site. Increasing [Ca$^{2+}$], also reduces the K$^+$ conductance of T cells (Bregestovski et al., 1986) and accelerates inactivation of K$^+$ channels of B cells (Choquet et al., 1987). Thus, both external and internal Ca$^{2+}$ regulate the effective number of K$^+$ channels.
The purpose of the experiments reported here is to define mechanisms of ion interaction with K⁺ channels. The similarity of effects when raising external or internal [Ca²⁺] suggests a common site of action. In some nerve preparations, inactivation of voltage-gated Ca²⁺ channels depends on Ca²⁺ entry through open Ca²⁺ channels and intracellular accumulation, rather than on membrane potential alone, a phenomenon known as Ca²⁺-dependent inactivation of the Ca²⁺ channel (for review see Eckert and Chad, 1984). An analogous mechanism in which sparingly permeant Ca²⁺ ions pass through the K⁺ channel and lead to inactivation might account for the effects of external di- and polyvalent cations on the rate of K⁺ channel inactivation described above. We therefore investigated whether the model of Ca²⁺-dependent inactivation of Ca²⁺ channels could be also applied to K⁺ channel inactivation of human T lymphocytes. Our experiments suggest that rather than modifying a site accessible to intracellular Ca²⁺ buffers, divalent ions can become trapped inside K⁺ channels. Since [Ca²⁺], is known to rise shortly after mitogen addition, the interaction of ions within the K⁺ channel may be relevant to signal transduction mechanisms. Some of the results have been reported in preliminary communications (Grissmer and Cahalan, 1987, 1988).

METHODS

The experiments were carried out on single cells of a human T lymphocyte-derived cell line, Jurkat E6-1, using the whole-cell recording mode of the patch-clamp technique (Hamill et al., 1981). All experiments were done at room temperature (22–26°C).

Solutions

The cells under investigation were bathed in normal Ringer solution. External solutions containing varying concentrations of Ca²⁺, Ba²⁺, K⁺, and Rb⁺ are listed in Table I. The chamber volume contained ~250 μl of solution which could be totally exchanged during the recordings by bath perfusion within 15–20 s. The patch-pipette usually contained 160 mM K-aspartate with [Ca²⁺] buffered to 10⁻⁸ M (see Table I). In some experiments the pipette solution contained higher [Ca²⁺].

Data Acquisition

The holding potential was adjusted in all experiments to E = -80 mV. The patch-clamp amplifier (either List L/M-EPC 7, Adams and List Associates, Ltd., Great Neck, NY; or Axon Instruments, Inc., Axopatch, Burlingame, CA) was used in the voltage-clamp mode without series resistance compensation. Electrodes were pulled from Accufill 90 Micropets (Becton, Dickinson & Co., Parsippany, NJ) in three stages, coated with Sylgard (Dow Corning Corp., Midland, MI) and fire polished to resistances, measured in the bath, of 2–7 MΩ. K⁺ channel inactivation was not different if hard glass was used as pipette glass (compare Cota and Armstrong, 1988; Furman and Tanaka, 1988).

In all experiments, the command input of the patch-clamp amplifier was controlled by a computer (PDP 11/73) via a digital-analog converter, and membrane currents were recorded at a bandwidth of 2 kHz. Correction for linear leakage and capacitative currents was achieved by analog subtraction and by digital subtraction of an appropriately scaled mean current associated with eight pulses delivered from a hyperpolarized potential. All potentials were corrected for the liquid junction potential that develops at the tip of the pipette if the pipette solution is different from that of the bath. The liquid junction potential between the normal internal (pipette) and external (bath) solution was -13 mV.
### Table 1

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The measured osmolarity of the solutions was 290–320 mosmol. Asp⁺, aspartate-; HEPES, N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid; EGTA, ethylene glycol-bis(β-aminoethyl ether)N,N,N',N'-tetraacetic acid. [Ca²⁺]ᵢ was calculated assuming a dissociation constant for EGTA and Ca²⁺ at pH 7.2 of 10⁻⁷ M according to Portzehl et al., 1964; in some experiments the intracellular Ca²⁺ buffering was increased by including 55 mM 1,2-bis(2-aminophenoxy)-ethane-N,N',N',N'-tetraacetic acid (BAPTA) and 5 mM CaCl₂ (free [Ca²⁺]ᵢ - 10⁻⁸ M) in the pipette solution. All concentrations are in millimolar.

### Analysis

Exponentials were fitted to the decay of the K⁺ current as described in more detail by Cahalan et al. (1985) by inputting values through potentiometers. Attempts to fit the decay of the K⁺ current in high [K⁺]₀ with a single exponential failed to describe the time course of
the K⁺ channel inactivation sufficiently. The fit, however, was improved by including a certain amount of baseline offset. This is consistent with the idea that there are K⁺ channels that do not inactivate during a depolarizing pulse, or that there is always a certain number of open K⁺ channels during depolarizations due to an equilibrium between the rate of activation and inactivation. Even with this fitting procedure, including an offset, the decay of the K⁺ current could not be adequately characterized, especially during the first 300–500 ms after the onset of the depolarizing pulse. Therefore, the decay of the K⁺ current in high [K⁺]o was described with a double exponential function.

RESULTS

External Ca²⁺ and Ba²⁺ Enhance Inactivation

Raising [Ca²⁺]o reversibly increases the rate of K⁺ channel inactivation in Jurkat cells, as illustrated by K⁺ currents in Fig. 1. Raising [Ca²⁺]o from 2 to 12 mM Ca²⁺ consistently reduced the peak current amplitude (I_{Kpeak}, from 1,314 to 1,200 pA in the cell of Fig. 1), as well as speeding the K⁺ current decay. The values for the inactivation time constants τ_h, obtained by single exponential fitting (see Methods), in normal Ringer solution before and after the Ca²⁺ treatment were 230 and 226 ms, respectively; τ_h in Ringer solution with 12 mM Ca²⁺ was 129 ms. While I_{Kpeak} in 12 mM Ca²⁺ Ringer is reduced to ~90% of the control value, τ_h is almost 1.8 times faster than in normal Ringer solution. Adding external Ba²⁺ results in similar effects on the time course of inactivation, but also induces a long-lasting “use-dependent” block of K⁺ currents, as described below.

Raising [Ca²⁺]o may change the negative surface potential of the cell by neutralizing surface charges, thereby shifting the voltage dependence of K⁺ channel gating to more depolarized potentials. To assess the surface potential effect, I_{K(E)} curves were measured and the corresponding g_{K(E)} curves were calculated. These two curves are shown in Fig. 2, A and B. The g_{K(E)} curve in 12 mM Ca²⁺ is shifted by 12 mV to more depolarized potentials, and g_{Kmax} is reduced to 93% of the control values before and after the Ca²⁺ treatment.

To quantify the influence of raising [Ca²⁺]o on the inactivation of the K⁺ channel, we measured K⁺ currents associated with pulses of different amplitudes. The inactivation time constants, τ_h, shown in Fig. 3 were slightly voltage dependent in normal Ringer, confirming earlier measurements on human T lymphocytes (see Fig. 6 of Cahalan et al., 1985). Raising Ca²⁺ shifts the voltage dependence of τ_h by an
A

The lines through the points were fitted with the Boltzmann equation:

\[ g_K(E) = g_{K,\text{max}}/[1 + \exp \left( \frac{(E_\text{m} - E)}{k} \right)] \]

with the following parameters:

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<th>During Ca\textsuperscript{2+} treatment</th>
<th>After</th>
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<td>10.59</td>
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<tr>
<td>( E_{\text{m}} ) (mV)</td>
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<td>( k ) (mV)</td>
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Same cell as in Fig. 1.

B

FIGURE 2. Shift in K\textsuperscript{+} channel activation in 12 Ca\textsuperscript{2+} Ringer. (A) Current-voltage relations before (○), during (△), and after (X) application of 12 Ca\textsuperscript{2+} Ringer. [Ca\textsuperscript{2+}]\textsubscript{i} = 10\textsuperscript{-8} M. Peak K\textsuperscript{+} currents obtained with 2-s depolarizing pulses are plotted against the absolute membrane potential, E, in mV. The lines through the points were drawn by eye for clarity. (B) Peak K\textsuperscript{+} conductance-voltage relations before (○), during (△) and after (X) application of 12 Ca\textsuperscript{2+} Ringer.

FIGURE 3. Inactivation time constants \( \tau \) of K\textsuperscript{+} currents before (○), during (△), and after (X) application of 12 Ca\textsuperscript{2+} Ringer. [Ca\textsuperscript{2+}]\textsubscript{i} = 10\textsuperscript{-8} M. Pulses were given every minute to allow complete recovery from inactivation. The time constants were obtained by fitting a single exponential to the decay of the K\textsuperscript{+} currents. The \( \tau \) values measured in 12 Ca\textsuperscript{2+} for a shift along the voltage axis determined from the shift of the conductance-voltage curve in 12 Ca\textsuperscript{2+} compared with normal Ringer solution. The lines through the points were drawn by eye for clarity. Same cell as in Figs. 1 and 2.
amount comparable to the shift of activation shown in Fig. 2. In addition to this shift, $\tau_h$ is reduced in 12 mM Ca\(^{2+}\)-Ringer. The combination of both effects causes a crossover of the control curve and the curve obtained in 12 mM Ca\(^{2+}\). To correct for surface potential effects, the $\tau_h$ values obtained in 12 mM Ca\(^{2+}\) (labeled “12 Ca uncorrected”) were shifted to more hyperpolarized potentials by the same amount that the $g_K(E)$ curve was shifted to more depolarized potentials (12 mV) when [Ca\(^{2+}\)]\(_o\) was raised from 2 to 12 mM (“12 Ca corrected”). The reduction of $\tau_h$ was then determined by calculating the ratios of the control $\tau_h$ and the corrected $\tau_h$ in 12 mM Ca\(^{2+}\). At $E = 10$ mV the reduction was to 52% of the control value, whereas at $E = 50$ mV the reduction was to 57% of the control value. This means that, aside from a shift, the Ca\(^{2+}\) effect on the inactivation time constant shows very little, if any, voltage dependence.

**Figure 4.** The change $p$ of the inactivation time constant $\tau_h$ in varying concentrations of Ca\(^{2+}\) (C) or Ba\(^{2+}\) (L). All external solutions contain 4.5 mM [K\(^{+}\)]\(_o\) [Ca\(^{2+}\)] = $10^{-9}$ M. $p$ is the normalized change of $\tau_h$, defined as

$$p = \frac{\tau_h - \tau_{h_{\text{min}}}}{\tau_{h_{\text{max}}} - \tau_{h_{\text{min}}}}$$

with $\tau_{h_{\text{max}}} = 430$ ms and $\tau_{h_{\text{min}}} = 65$ ms. Divalent ion concentration [X\(^{2+}\)]\(_o\) is plotted on a logarithmic scale. The bars on the points reflect the standard deviation, each point reflects the mean of at least three measurements. The curve through the points was calculated assuming that $p$ is proportional to $K_d/([X^{2+}]_o + K_d)$ with $K_d = 3.5$ mM for Ca\(^{2+}\), and $K_d = 1.5$ mM for Ba\(^{2+}\).

Fig. 4 shows the change in $\tau_h$ ($p$, as defined in the legend) in Ringer solutions with different [Ca\(^{2+}\)]\(_o\) or [Ba\(^{2+}\)]\(_o\). The results indicate that raising [Ca\(^{2+}\)]\(_o\) from $10^{-9}$ M to 100 mM accelerates the rate of inactivation more than fivefold. The change is normalized such that $p = 1$ corresponds to the maximum inactivation time constant, $\tau_{h_{\text{max}}} = 430$ ms; $p = 0$ corresponds to the minimum inactivation time constant, $\tau_{h_{\text{min}}} = 65$ ms. The estimate of $\tau_{h_{\text{max}}}$ was obtained by measuring $\tau_h$ in Ringer solutions with [Ca\(^{2+}\)]\(_o\) = $10^{-9}$ M and it was found to be $422 \pm 17$ ms ($n = 3$), whereas $\tau_{h_{\text{min}}}$ was determined by fitting the calculated curve to the measured data points. $\tau_{h_{\text{max}}}$ was the same for either Ca\(^{2+}\) or Ba\(^{2+}\). It is concluded that Ca\(^{2+}\) or Ba\(^{2+}\) can bind with apparent $K_d$'s of 3.5 and 1.5 mM to a site with one-to-one stoichiometry (Hill plots of these curves yield a slope of 1.03 and 1.01, respectively), and thus influence K\(^{+}\) channel inactivation.
In addition to accelerating the rate of inactivation, $\text{Ba}^{2+}$ ions result in progressive "use-dependent" block of $K^+$ current. Fig. 5A shows a $K^+$ current in normal Ringer associated with a 1-s test pulse to 40 mV from a holding potential of $-100$ mV. Immediately after recording this trace, the bath solution was changed to 10 mM $\text{Ba}^{2+}$ and $K^+$ currents elicited by identical test pulses were recorded. The first trace in 10 mM $\text{Ba}^{2+}$ shows an acceleration in the decay of the $K^+$ current, whereas the peak current amplitude is hardly affected, which is similar to the effect of raising external $\text{Ca}^{2+}$ shown in Fig. 1. Subsequent test pulses result in a progressive reduction of the peak current amplitude. This type of "use-dependent block" is similar to the effects of external $\text{Ba}^{2+}$ on the delayed outward $K^+$ currents in muscle fibers (Werman and Grundfest, 1961; Sperelakis et al., 1967), squid axons (Armstrong and Taylor, 1980), and myelinated nerve fibers (Arhem, 1980). The peak $K^+$ current amplitudes are plotted in Fig. 5B. A single exponential fit to these points yields an apparent time course for blocking the $K^+$ channels by $\text{Ba}^{2+}$. This apparent time course strongly depends on the interval between test pulses (data not shown), suggesting that $\text{Ba}^{2+}$ can block only if the channel is open.

Fig. 6 illustrates that removal of the $\text{Ba}^{2+}$ block also depends on channel opening. After changing the bath solution from 10 mM $\text{Ba}^{2+}$ to normal Ringer solution (shown at the arrow), the $\text{Ba}^{2+}$ block of $K^+$ current is slowly removed as the depolarizing pulses are continued; peak $K^+$ current recovers almost to the original level, $\sim 1,400$ pA. The time constant for washing out the $\text{Ba}^{2+}$ using this particular protocol was $\sim 80$ s. If, on the other hand, pulses are not delivered after washing out the $\text{Ba}^{2+}$, the channels remain blocked, as illustrated in the right-hand side of the figure. This time, after establishing the $\text{Ba}^{2+}$ block in 10 mM $\text{Ba}^{2+}$ and then restoring the
bath solution to normal Ringer solution, the membrane potential was held constant at \(-80\) mV for 10 min without pulses. When the same sequence of depolarizing pulses was then delivered, the time course for washing out the \(\text{Ba}^{2+}\) was the same as the one on the left side, except for the 10-min delay in which channels remained closed. \(\text{Ba}^{2+}\) can therefore be trapped within the closed channel and cannot dissociate from the blocking site until after the channel opens. The time course for washing out the \(\text{Ba}^{2+}\) also depends very strongly on the interval between pulses (data not shown), suggesting that the longer the channels are held open, the faster the washout. This mechanism is similar to \(\text{Ba}^{2+}\) block of delayed outward \(\text{K}^+\) current in squid axons and myelinated nerve fibers (Armstrong and Taylor, 1980; Eaton and Brodwick, 1980; Armstrong et al., 1982; Woll, 1982) and of voltage-dependent \(\text{Ca}^{2+}\)-activated \(\text{K}^+\) channels from skeletal muscle (Vergara and Latorre, 1983; Miller, 1987; Miller et al., 1987).

**Internal \(\text{Ca}^{2+}\) Mimics External \(\text{Ca}^{2+}\)**

Bregestovski et al. (1986), as well as Choquet et al. (1987), suggested that internal \(\text{Ca}^{2+}\) may play a role in regulating the number of \(\text{K}^+\) channels capable of being activated. In further experiments, we increased the intracellular \(\text{Ca}^{2+}\) concentration and monitored the inactivation time constant, \(\tau_h\), at \(+40\) mV after breaking into the cells with pipettes filled with either \([\text{Ca}^{2+}]_i = 10^{-8}\) M, \(10^{-5}\) M, or \(2\) mM, as illustrated in Fig. 7. With pipettes containing \(10^{-8}\) M \(\text{Ca}^{2+}\) (squares) there is a small reduction of \(\tau_h\) 10 min after breaking into the cell. This is probably due to the grad-

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**Figure 6.** Removal of the \(\text{Ba}^{2+}\) block. \(\text{K}^+\) currents were elicited with the same pulse protocol as described (legend of Fig. 5), and peak \(\text{K}^+\) currents \((I_{\text{peak}})\) were plotted at different times after the solution change from normal Ringer to 10 mM \(\text{Ba}^{2+}\). \([\text{Ca}^{2+}]_i = 10^{-8}\) M. The bath solutions were changed from 10 mM \(\text{Ba}^{2+}\) to normal Ringer solution at the arrows. (A) The block of the \(\text{K}^+\) current is removed without delay, and the peak \(\text{K}^+\) current recovers almost to the original level before the \(\text{Ba}^{2+}\) treatment if depolarizing pulses are given every 53 s. Cell 604. (B) No recovery of \(I_{\text{peak}}\) occurred if the membrane was not depolarized. After 10 min in normal Ringer solution the membrane was depolarized with the same pulse protocol used for monitoring the \(\text{Ba}^{2+}\) washout in A. Cell 603.
ual disappearance of a junction potential between the pipette solution and the cytoplasm (Marty and Neher, 1983). When the patch pipettes were filled with $[\text{Ca}^{2+}]_i = 10^{-5}$ M, there was a much more pronounced effect of $\tau_h$ after breaking into the cell (triangles). After 10 min, the $\tau_h$ values with $[\text{Ca}^{2+}]_i = 10^{-5}$ M correspond to a 1.7-fold increase in the rate of inactivation, which was somewhat less than the threefold decrease in half-decay time reported by Choquet et al. (1987), and possibly due to the different test pulse voltages used. If the patch pipettes contained 2 mM $[\text{Ca}^{2+}]_i$, inactivation accelerated even further after breaking into the cell (circles). After 5 min, the $\tau_h$ values with $[\text{Ca}^{2+}]_i = 2$ mM corresponded to a fivefold increase in the rate of inactivation. With such a high internal Ca$^{2+}$ concentration, it was impossible to record longer than 6 min after breaking into the cell.

If the binding site for Ca$^{2+}$ that influences the rate of K$^+$ channel inactivation is located within the membrane field, and Ca$^{2+}$ is able to reach this site from either side of the membrane, raising $[\text{Ca}^{2+}]_i$ may alter the voltage dependence of the effect of $[\text{Ca}^{2+}]_o$ on $\tau_h$. To assess this, we did experiments of the type shown in Figs. 1–3, but with $[\text{Ca}^{2+}]_i = 10^{-5}$ M. The $\tau_h$ values obtained in normal Ringer with $[\text{Ca}^{2+}]_i = 10^{-5}$ M were plotted against the membrane potential, $E$, as shown in Fig. 8 (open squares). Raising $[\text{Ca}^{2+}]_o$ from 2 to 12 mM with $[\text{Ca}^{2+}]_i = 10^{-5}$ M shifted the $I_h(E)$ and $g_h(E)$ curves of the K$^+$ current by 10 mV to more depolarized potentials (not
shown). Therefore, it was necessary to correct the $\tau_\text{h}$ values measured with $[\text{Ca}^{2+}]_o = 12 \text{ mM}$ and $[\text{Ca}^{2+}]_i = 10^{-5} \text{ M}$ (filled triangles), in a manner similar to that shown in Fig. 3. The reduction of $\tau_\text{h}$ with normal Ringer outside and the corrected $\tau_\text{h}$ in Ringer with 12 mM Ca$^{2+}$ outside. At $E = 10 \text{ mV}$, the reduction was to 63% of the control value, whereas at $E = 50 \text{ mV}$ the reduction was to ~72% of the control value. This means that the effect of raising $[\text{Ca}^{2+}]_o$ on the inactivation time constant shows little voltage dependence. Comparing the effect of increased $[\text{Ca}^{2+}]_o$ on $\tau_\text{h}$ with $[\text{Ca}^{2+}]_i = 10^{-5}$ or $10^{-6} \text{ M}$ yields two results. First, the voltage dependence of increased $[\text{Ca}^{2+}]_o$ on the inactivation time constant is steeper with $[\text{Ca}^{2+}]_i = 10^{-5} \text{ M}$ compared with $10^{-6} \text{ M}$. Second, the change in $\tau_\text{h}$ produced by raising $[\text{Ca}^{2+}]_o$ is smaller when $[\text{Ca}^{2+}]_i = 10^{-5} \text{ M}$ compared with $10^{-6} \text{ M}$, which suggests competition between $[\text{Ca}^{2+}]_i$ and $[\text{Ca}^{2+}]_o$ for the same site. In addition, this series of experiments provides further evidence that the lymphocyte K$^+$ channel is not activated by intracellular Ca$^{2+}$, as we did not observe any significant shift of the activation parameters to more hyperpolarized potentials with $[\text{Ca}^{2+}]_i = 10^{-5} \text{ M}$, compared with $[\text{Ca}^{2+}]_i = 10^{-6} \text{ M}$.

If there is a binding site located on the cytoplasmic side of the membrane, which can be accessed by Ca$^{2+}$ ions from the outside moving through the channel, then increasing the intracellular Ca$^{2+}$ buffering may reduce the effect of extracellular Ca$^{2+}$, by analogy with Ca$^{2+}$-dependent inactivation of Ca$^{2+}$ channels (Eckert and Chad, 1984). However, increasing cytoplasmic Ca$^{2+}$ buffering by including 55 mM BAPTA and 5 mM Ca$^{2+}$ in the pipette (free $[\text{Ca}^{2+}]_i = 10^{-8} \text{ M}$) did not change the effect of Ca$^{2+}$ on $\tau_\text{h}$ (data not shown). These results suggest either that the site at which $[\text{Ca}^{2+}]_o$ binds is inside the K$^+$ channel and not accessible to cytoplasmic Ca$^{2+}$ buffers, or that there are two sites, one external and one internal.

**K$^+$ and Ca$^{2+}$ Have Opposing Effects on K$^+$ Channel Inactivation**

Reducing $[\text{K}^+]_o$ from 4.5 to 0 mM has almost the same effect on the K$^+$ current as does increasing $[\text{Ca}^{2+}]_o$, as shown in Fig. 9 A. The time course of the decay of the
K⁺ current is faster in 0 mM K⁺ compared with normal Ringer solution, with \( \tau_h \) values of 200 and 285 ms, respectively. Thus, removing K⁺ from the Ringer accelerated the rate of inactivation by a factor of 1.4. Fig. 9B illustrates the effect of increasing \([K⁺]_o\) from 4.5 to 160 mM. The reduction of the K⁺ current amplitude in high \([K⁺]_o\) is due to a change in reversal potential \(E_k\) for potassium ions; thus, the driving force of these ions at +40 mV is reduced. In addition to slowing channel closing during tail currents (Cahalan et al., 1985), raising \([K⁺]_o\) slows down inactivation. The time course of the inactivation in 160 mM K⁺ Ringer could be well described by a double exponential with fast and slow inactivation time constants, \(\tau_{hf}\) and \(\tau_{hs}\). The value for \(\tau_{hf}\) was 320 ms and was 1.6 times larger than the \(\tau_h\) value found in normal Ringer solution (200 ms). The value for \(\tau_{hs}\) was ~4 s, a slow inactivation not seen in normal Ringer.

A dose-response curve demonstrating the effect of \([K⁺]_o\) on K⁺ channel inactivation is shown in Fig. 10. The change in the fast inactivation time constant was normalized as was done previously with Ca²⁺. A value of 1 corresponds to the maximum inactivation time constant of 430 ms. This \(\tau_h\) value was assumed to be the same as in the experiments with \([Ca²⁺]_o = 10^{-9} \text{ M}\). A value of 0 corresponds to the inactivation time constant of 200 ms and was obtained in experiments with \([K⁺]_o = 0 \text{ mM}\) as shown in Fig. 9A. Fits of these data to the Hill equation yielded a slope of 1.1, which is consistent with the idea that only a single potassium ion is required to slow \(\tau_{hf}\). A calculation of an adsorption isotherm to the dose-
response curve (line in Fig. 10) is in agreement with the data with a value of 13 mM for the apparent dissociation constant of $K^+$ at its site of action.

Three explanations for the opposing influences of $Ca^{2+}$ and $K^+$ on inactivation are possible. (a) Two different receptors at the $K^+$ channel interact allosterically; $Ca^{2+}$ bound to its receptor results in faster inactivation and $K^+$ bound to its receptor results in a loss of affinity of the $Ca^{2+}$ receptor for $Ca^{2+}$. (b) $Ca^{2+}$ can bind to a site where it can speed inactivation, and $K^+$ either competes with $Ca^{2+}$ for this site or prevents $Ca^{2+}$ from reaching it. (c) There are two separate and independent binding sites for $Ca^{2+}$ and $K^+$; $Ca^{2+}$ binding will result in a faster inactivation, whereas $K^+$ binding will result in slower inactivation. The agreement of $\tau_{\text{hmax}}$ with $[Ca^{2+}]_o = 10^{-9}$ M and of $\tau_{\text{hmax}}$ with $[K^+]_o = 160$ mM (compare Figs. 4 and 10) argues against this idea of independent sites. Table II shows calculations of apparent dissociation constants of the $Ca^{2+}$ site reaction (a) for the case of an affinity change at the $Ca^{2+}$ binding site and (b) for the case of competition. Values of $K_d$, reflecting the change in affinity of the site for $Ca^{2+}$ under (a), change almost two orders of magnitude (compare $K_d = 1$ mM for $[K^+]_o = 0$ mM with $K_d = 79$ mM for $[K^+]_o = 160$ mM).

This change in $K_d$ seems to be too large to be explained by a loss in affinity of the $Ca^{2+}$ binding site for $Ca^{2+}$. For the case of competition (b), the calculated dissociation constant of $Ca^{2+}$ and its receptor, in the presence of the competing $K^+$, should be independent of $[K^+]_o$, as indicated in Table II assuming that the true dissociation constant for the reaction of $K^+$ with the receptor, i.e., $K_K$, is $\sim 2$ mM. From these calculations we favor the idea that $Ca^{2+}$ and $K^+$ compete for the same binding site.

**External Rb$^+$ and Divalent Trapping**

The effects of replacing NaCl and KCl in normal Ringer with RbCl were similar to those shown in Fig. 9 B in which $[K^+]_o$ is increased from 4.5 to 160 mM. Interestingly, however, the first trace, after the application of Rb$^+$-Ringer, was consistently different in the time course of activation from the second and following traces in Rb$^+$-Ringer, as illustrated in Fig. 11 A. One can see that the $K^+$ current associated with the first test pulse to +40 mV in Rb$^+$-Ringer activates very rapidly (within 5 ms) to 767 pA, then activates further very slowly to reach a peak after 100 ms. It
then inactivates with a time course similar to the slow inactivation seen in 160 mM K\(^{+}\)-Ringer. The K\(^{+}\) currents associated with the second and following test pulses in Rb\(^{+}\)-Ringer do not show the slow activation; the peak K\(^{+}\) current is reached within 10 ms. The K\(^{+}\) current decays associated with the first, second, and subsequent test pulses in Rb\(^{+}\)-Ringer are superimposable. In addition, tail currents in Rb\(^{+}\)-Ringer are prolonged compared with normal, as observed previously (Cahalan et al., 1985). We believe that the slow phase of activation represents the removal of divalent ions from K\(^{+}\) channels, for reasons described below. On this assumption, the difference between the first and second current traces after applying Rb\(^{+}\)-Ringer represents the time course of unblocking. The peak amplitude of the difference current was 20% of the amplitude of the scaled second current trace, and the time course of the decay of this difference current was 72 ms. In a total of five experiments we obtained a relative peak amplitude of the difference current of 24 \(\pm\) 3% (mean \(\pm\) SD); the time constant of the decay of these difference currents was 75 \(\pm\) 6 ms.

Pretreatment with 10 mM BaCl\(_2\) increased the amplitude of the difference cur-

### Table II

**Calculated Dissociation Constant, \(K_{d}\), of Ca\(^{2+}\) Binding Site**

<table>
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<tr>
<th>([\text{Ca}^{2+}]_0) mM</th>
<th>([\text{K}^{+}]_0) mM</th>
<th>(n)</th>
<th>(K_{d}) calculated for assumptions</th>
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<th>(\theta)</th>
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Apparent dissociation constant, \(K_{d}\), of Ca\(^{2+}\) site reaction calculated for the case of (a) exclusive change of affinity of the Ca\(^{2+}\) binding site by K\(^{+}\), and for the case of (b) competition. The equations used are from Ulbricht and Wagner (1975) and Grissmer (1984) with little modification. The equations are:

\[ *K_{d} = \frac{[\text{Ca}^{2+}]_0}{(1/p - 1)} \]

\(p\) is defined in legend of Fig. 4 and

\[ 1^{K_{d}} = \frac{[\text{Ca}^{2+}]_0}{(1/p - 1)(1 + [\text{K}^{+}]_0/K_{x})} \]

\(K_{x}\) is the dissociation constant for the reaction of K\(^{+}\) with the Ca\(^{2+}\) binding site, assuming that there is no direct influence of K\(^{+}\) on the fast inactivation with \(K_{x} = 2\) mM.
rent between the first and second current traces in Rb⁺-Ringer to 76% of the peak, without changing the time constant of the decay of this difference current (70 ms), compared with that without Ba²⁺ pretreatment. This result, illustrated in Fig. 11 B, indicates that the fraction of channels containing trapped divalent ions is correlated with the amplitude of the difference current between the first and second current traces in Rb⁺-Ringer. It also suggests that the relative amplitude of the difference current under normal conditions (i.e., no Ba²⁺ pretreatment) reflects the percentage of channels that have Ca²⁺ trapped inside.

To test whether the fraction of channels with trapped Ca²⁺ can be modified, we varied external and internal [Ca⁺]. Increasing [Ca²⁺]o to 106 mM Ca²⁺ accelerated inactivation, but changed neither the relative amplitude of the difference current

![Figure 11](https://rupress.org/jgp/article-pdf/93/4/609/599069/609.pdf)

**Figure 11.** K⁺ currents associated with the first and second 2-s depolarizing test pulses to +40 mV (holding potential -80 mV) after changing to Rb⁺-Ringer. Pulse interval was 53 s. The bath solution was totally exchanged before delivering the pulses. (Upper row) Bath solution was changed from normal Ringer to Rb⁺-Ringer ([Ca²⁺] = 10⁻⁸ M) in A, from a Ringer solution with 10 mM Ba²⁺ to Rb⁺-Ringer ([Ca²⁺] = 10⁻⁸ M) in B, and from normal Ringer to Rb⁺-Ringer ([Ca²⁺] = 10⁻⁹ M) in C; records were taken after the solution change. (Lower row) Difference current of the first and second current trace in Rb⁺-Ringer. Between the first and second current traces in Rb⁺-Ringer nor its decay. This suggests that Ca²⁺ loaded from the outside during the pulse may have been released to the inside during the 1-min pulse interval between the last current trace in 106 mM Ca²⁺ and the first trace in Rb⁺-Ringer. This led us to attempt to determine whether we could increase the number of channels that have Ca²⁺ trapped inside by increasing [Ca²⁺]. For this purpose we used patch-pipettes filled with [Ca²⁺]₀ = 10⁻⁵ M. After breaking into the cell, we waited at least 5 min to attain equilibrium between the pipette solution and the cell cytoplasm. The pipette solution was then changed to Rb⁺-Ringer. The difference between the first and second current trace obtained under these conditions is shown in Fig. 11 C. The peak amplitude of the difference current was 57% of the second current trace; the decay of the difference current
was 71 ms. In a total of five experiments, we obtained 52 ± 8% for the relative amplitude of the difference current; the time course for the decay of these difference currents was 76 ± 4 ms. We conclude that it is possible to load the channels with Ca\(^{2+}\) from the inside, and that this Ca\(^{2+}\) can be released to the outside.

**Effects of Removing All Divalent Cations**

To see whether the residual inactivation rate of K\(^+\) channels (which corresponds to \(\tau_{\text{hmax}}\) in low external and internal \([Ca^{2+}]\) is due to block by Mg\(^{2+}\) ions, which would be analogous to the Mg\(^{2+}\) block of inward rectifier K\(^+\) channels (Matsuda et al., 1987; Vandenberg, 1987), experiments were performed using internal solutions with no added Mg\(^{2+}\) \([Ca^{2+}] = 10^{-8} \text{ M}\). After breaking into a cell we recorded normal inactivating K\(^+\) currents even after 15 min with normal Ringer outside. If we changed to a Ringer solution with no added Mg\(^{2+}\) and \([Ca^{2+}]_o = 10^{-9} \text{ M}\), changes in the membrane current that were similar to those described by Armstrong and Lopez-Barneo (1987) occurred. We observed the following results. (a) The inward holding current increased. (b) When the voltage was stepped to \(E = +40 \text{ mV}\) from a holding potential of \(-80 \text{ mV}\), there was an instantaneous jump in current from inward to outward. (c) The K\(^+\) current became smaller, with slower decay of the current (comparable with the experiments with \([Ca^{2+}]_o = 10^{-9} \text{ M}\), and disappeared at last. Extending these results, which are similar to those found previously on squid neurons (Armstrong and Lopez-Barneo, 1987), we found that: (d) the induction of the new “leakage” conductance could be prevented by 2 mM Mg\(^{2+}\) inside. (e) This conductance could be blocked by either 1 mM Ca\(^{2+}\) or 1 mM Mg\(^{2+}\) outside. (f) Ba\(^{2+}\) could not only prevent the induction of this conductance but could also block it after its development with a \(K_d\) around 3 mM. (g) The induction of this conductance could not be prevented by totally blocking the K\(^+\) channels with 10 nM charybdotoxin, a potent blocker of the K\(^+\) channel (Sands et al., 1988), either before or during treatment with zero-divalent external and internal solutions. Although raising \([Mg^{2+}]_o\) to 12 mM has little effect on the K\(^+\) channel inactivation rate (data not shown), even 1 mM Mg\(^{2+}\) can “protect” the K\(^+\) channels while also preventing the increased leakage current seen in zero divalent. We are uncertain whether this conductance is related to the K\(^+\) channel protein, or, alternatively, represents a nonspecific conductance induced by zero divalent.

**DISCUSSION**

It is well established that ions can alter the gating of voltage-dependent channels. One mechanism involves electrostatic attraction and binding of ions to negative surface charges at each side of the membrane (for review, see Hille, 1984), thereby influencing the voltage dependence of channel gating. Several observations, however, cannot be explained by surface potential theory alone. One example is that externally applied Zn\(^{2+}\) ions slow activation without altering the time course of deactivation in squid axons (Gilly and Armstrong, 1982). State-dependent binding mechanisms, in which the ion binds more strongly to a particular channel conformation, provide an explanation for effects of divalent ions on channel gating. A special case of state-dependent mechanisms involves ion binding to open channels. For example,
occupancy of an open channel by either a permeant or blocking ion has been hypothesized to hinder channel closing for both acetylcholine receptors and K channels (Marchais and Marty, 1979; Swenson and Armstrong, 1981; Cahalan and Pappone, 1983; Matteson and Swenson, 1986; Armstrong and Matteson, 1986). The effects of permeant monovalent, divalent, and blocking ions on T cell K^+ channels are further examples of state-dependent channel mechanisms. A novel aspect of the results reported here is that we have focused on the effects of ions on channel inactivation gating.

**Divalent Effects on Inactivation: Block or Modulation**

We have shown that the lymphocyte K^+ channel inactivation rate is strongly influenced by external Ca^{2+} (or Ba^{2+}), as well as internal Ca^{2+}. There are two possible mechanisms that should be considered. Ca^{2+} binding to a site in the channel may result in (or modulate the rate of) a conformational change that inactivates the channel. A second possibility is that block of the K^+ channel by a Ca^{2+} ion is the inactivation mechanism. In this case, depolarization to open the channel would also enable entry and block by divalent ions; the slow recovery from inactivation would represent the unbinding of trapped Ca^{2+}. In either mechanism, if Ca^{2+} entry were decreased (a) by reducing \([Ca^{2+}]_o\), (b) by competition with an external permeant ion, or (c) by a K^+ channel blocker, then inactivation should become slower. We now consider experimental evidence on each of these points. (a) As \([Ca^{2+}]_o\) is reduced, the time constant of inactivation approaches a maximum value, \(t_{inh} \approx 430\) ms. This residual inactivation observed in essentially Ca^{2+}-free internal and external solutions may be due to an intrinsic inactivation mechanism, or due to block by another ion present, for example Mg^{2+}. The induction of "leak" conductance and disappearance of K^+ conductance upon the removal of all divalents prevents a clear test of this hypothesis. (b) When \([K^+]_o\) is elevated, inactivation becomes slower and incomplete, even during pulses lasting 4 s (Fig. 9 B). Table II describes the interaction between \([K^+]_o\) and \([Ca^{2+}]_o\) on the fast component of inactivation; permeant monovalent ions may compete with Ca^{2+} for entry into the channel. A slow component of inactivation with a time constant greater than \(t_{inh} \approx 430\) ms is revealed when \([K^+]_o\) is raised. This may indicate that \(t_{inh} \approx 430\) ms is not due to an intrinsic inactivation mechanism. (c) External tetraethylammonium ([TEA^+]_o) slows the time constant of inactivation as it reduces the current, resulting in crossover of the K^+ currents in the presence and absence of TEA^+ and a constant current integral during long depolarizing pulses as [TEA^+]_o is elevated (Grissmer and Cahalan, 1989). Similar crossover of K^+ current due to channel block, combined with slowing of inactivation, was also observed with external Mn^{2+} or Co^{2+} ions (DeCoursey et al., 1985). The kinetic effects of TEA^+ on inactivation were modeled by a scheme in which channels blocked by TEA^+ cannot inactivate (Grissmer and Cahalan, 1989). TEA^+ (or Mn^{2+} or Co^{2+}) blocking the K^+ channel may prevent inactivation by preventing Ca^{2+} entry. If this is true, then the fact that TEA^+ results in \(t_{inh} \approx 430\) ms, as discussed in (a) above, and in favor of divalent entry being a prerequisite for inactivation. Either divalent block or a conformational change induced by a divalent ion within the channel would be consistent with the evidence described above.
The concentration dependence of the Ca$^{2+}$ (or Ba$^{2+}$) effect on inactivation is apparently inconsistent with a simple divalent-block hypothesis; $t_{\text{min}}$ saturates at a minimum value of 65 ms as [Ca$^{2+}$]$_o$ or [Ba$^{2+}$]$_o$ is elevated. According to a simple divalent-block mechanism for inactivation, one would expect the time constant of inactivation to decrease monotonically as the concentration of divalent is raised, as is the case for internal Ba$^{2+}$ block of squid axon K$^+$ channels (Armstrong and Taylor, 1980; Eaton and Brodwick, 1980; Armstrong et al., 1982). The effects of both [Ca$^{2+}$]$_o$ and [Ba$^{2+}$]$_o$ on lymphocyte K$^+$ channel inactivation can be described by a simple 1:1 binding isotherm, with saturation at high [Ca$^{2+}$]$_o$ or [Ba$^{2+}$]$_o$ at the same value of $t_{\text{min}}$ (Fig. 4). This result suggests that divalent binding to a site inside the channel induces a conformational change that inactivates the channel with a limiting rate corresponding to $t_{\text{min}}$. A more complicated blocking model involving extremely slow single filing of Ca$^{2+}$ (or Ba$^{2+}$) between an outermost saturable site and a blocking site within the channel might also account for $t_{\text{min}}$. Regardless of the exact mechanism, the acceleration of inactivation by divalent ions parallels the idea that channel closing (deactivation) is more rapid when channels contain Ca$^{2+}$ than when they contain monovalent ions, as proposed for squid axon K$^+$ channels (Armstrong and Matteson, 1986).

**Blocking Model for [Ca$^{2+}$]$_o$ and [Ca$^{2+}$]$_i$**

Raising either internal or external [Ca$^{2+}$] accelerates the rate of inactivation. Competition between internal and external Ca$^{2+}$ ions for the same binding site within the channel may result in the diminished effect of [Ca$^{2+}$]$_o$ on $\tau_n$ when [Ca$^{2+}$]$_i$ is raised (Figs. 3 and 8). We modeled the change in $\tau_n$ by assuming that Ca$^{2+}$ can block the open channel nearly irreversibly, and that Ca$^{2+}$ may reach the blocking site from either side of the membrane. Under this assumption one can separate $\tau_n$ due to an intrinsic inactivation process (measured in [Ca$^{2+}$]$_o$ = $10^{-9}$ M and [Ca$^{2+}$]$_i$ = $10^{-8}$ M) from $\tau_{\text{Ca}}$ due to Ca$^{2+}$ reaching the site. These $\tau_{\text{Ca}}$ values measured in different [Ca$^{2+}$]$_o$ and different [Ca$^{2+}$]$_i$ were plotted against the absolute membrane potential $E$ as shown in Fig. 12 (open squares). The voltage dependent of $\tau_{\text{Ca}}$ changes from a small increase of $\tau_{\text{Ca}}$ with more depolarization in [Ca$^{2+}$]$_i$ = $10^{-8}$ M, to a marked reduction of $\tau_{\text{Ca}}$ with depolarization in [Ca$^{2+}$]$_i$ = $10^{-5}$ M. The lines through the points are least-squares fits to the data considering that the K$^+$ channel has one local free energy minimum (site) that can either be empty or occupied by Ca$^{2+}$. Ca$^{2+}$ moves from either side of the membrane to the site over an intervening energy maximum (barrier). The data could be reasonably described by a free energy profile shown in Fig. 13, which illustrates a two-barrier one-site model. In this model the binding site for Ca$^{2+}$ is almost at the extracellular side of the membrane, perhaps near the channel mouth. The reason for the very broad plateau of the inner barrier is the weak voltage dependence of $\tau_{\text{Ca}}$ in all the solutions with different Ca$^{2+}$ concentrations. Alternatively, a second two-barrier one-site model, with the plateau at the outer barrier, is indistinguishable from the one shown in Fig. 13. The Ca$^{2+}$ binding site would then be about two thirds of the way across the electric field from the outside.

The similar effects raising internal and external [Ca$^{2+}$] on K$^+$ channel inactivation (Figs. 3 and 8), and the unblocking of divalent cations from K$^+$ channels (Fig. 11),
are results consistent with Ca\(^{2+}\) ions being sparingly permeant through K\(^{+}\) channels, as suggested by the energy profile of Fig. 13. A calculation of the Ca\(^{2+}\) flux through an open K\(^{+}\) channel using the rate constants for leaving the Ca\(^{2+}\) binding site (obtained from the barrier heights and well depth given in legend of Fig. 13) shows that this Ca\(^{2+}\) flux is \(\sim 0.6\) ions/channel per s at 0 mV. Even with all K\(^{+}\) channels open, the maximum Ca\(^{2+}\) flux in a human T cell with 400 channels would be \(\sim 250\) ions/cell per s, at least 2 orders of magnitude smaller than estimates of Ca\(^{2+}\) entry from tracer flux measurements which were reported by Metcalfe et al. (1980) and Hesketh et al. (1983) to be 24,000–130,000 Ca\(^{2+}\) ions/cell per s. The calculated flux is based upon the blocking model with a static energy profile as shown in Fig. 13. As discussed above, the changes in inactivation may result from modulation of an intrinsic inactivation process as Ca\(^{2+}\) ions from either side of the membrane bind to a site within the membrane. We cannot estimate the flux in this case.

In single channel studies of the Ca\(^{2+}\)-activated K\(^{+}\) channel, Neyton and Miller (1988, c) described Ba\(^{2+}\) block from either the cis or trans side of the membrane in terms of a single site within the channel that can bind Ba\(^{2+}\). In their model to account for interactions between Ba\(^{2+}\) and K\(^{+}\), three additional sites can bind K\(^{+}\) ions (Neyton and Miller, 1988c), but when [K\(^{+}\)]\(_{o}\) is lowered a second Ba\(^{2+}\) ion can also bind from the outside (C. Miller, personal communication). The asymmetric

\[\tau_{\text{Ca}}\] for Ca\(^{2+}\) reaching the site at the K\(^{+}\) channel at four different combinations of [Ca\(^{2+}\)]\(_{o}\) and [Ca\(^{2+}\)]\(_{i}\). (A) [Ca\(^{2+}\)]\(_{o}\) = 2 mM, [Ca\(^{2+}\)]\(_{i}\) = 10\(^{-8}\) M. (B) [Ca\(^{2+}\)]\(_{o}\) = 2 mM, [Ca\(^{2+}\)]\(_{i}\) = 10\(^{-5}\) mM. (C) [Ca\(^{2+}\)]\(_{o}\) = 12 mM, [Ca\(^{2+}\)]\(_{i}\) = 10\(^{-8}\) M. (D) [Ca\(^{2+}\)]\(_{o}\) = 12 mM, [Ca\(^{2+}\)]\(_{i}\) = 10\(^{-5}\) M. \(\tau_{\text{Ca}}\) was obtained by dividing the time course of the decay of the K\(^{+}\) current with [Ca\(^{2+}\)]\(_{o}\) = 10\(^{-9}\) M by the time course of the decay of the K\(^{+}\) current in the different solutions. The newly created time course reflects the time course of Ca\(^{2+}\) reaching its site at the channel. The lines through the points were least-squares fits to the data with values for the inner and outer barrier; the well and the electrical distances are given in legend of Fig. 13.
concentration dependence and rates of Ba$^{2+}$ block in the Ca$^{2+}$-activated K$^+$ channel are similar to the Ca$^{2+}$ effect on lymphocyte K$^+$ channel inactivation described here. Although a single-site model may account for the divalent effects on inactivation, we cannot rule out additional divalent binding sites.

**Trapping of Divalent Ions**

The data presented in Figs. 5, 6, and 11 illustrate some of the dynamics of divalent trapping in the K$^+$ channel. Fig. 5 demonstrates that Ba$^{2+}$ ions cannot enter the K$^+$ channel until it is opened. The channels must be opened in order for recovery from Ba$^{2+}$ block to occur (Fig. 6). The recovery rate strongly depends on the interval between test pulses, suggesting that if the channels could be held open longer, the recovery from Ba$^{2+}$ block would be faster. However, the channels inactivate during depolarizing pulses, and so Ba$^{2+}$ ions do not dissociate from all the channels during a single depolarizing pulse. If one could keep the channels open for a very long time by preventing inactivation one might see the real time course for Ba$^{2+}$ leaving the channel with a single depolarizing test pulse. We succeeded in reducing the rate of inactivation by changing to high Rb$^+$-Ringer, and we were able to measure the time course for Ba$^{2+}$ and Ca$^{2+}$ leaving the K$^+$ channel (see Fig. 11). The time course for leaving the K$^+$ channel, reflected in the decay of the difference current shown in Fig. 11, is the same for either Ba$^{2+}$ or Ca$^{2+}$. This similarity in the time course of the difference current may result from the dissociation of Rb ions being rate limiting; if Rb$^+$ binds to a site and can prevent the removal of a Ca$^{2+}$ or Ba$^{2+}$ ion, the time course of the difference current would be limited by the time course for Rb$^+$ leaving its site. This assumption could also explain the failure to observe a difference in the first and second current trace in 160 mM K$^+$ Ringer. Under normal conditions ~25% of all channels contain a Ca$^{2+}$ ion. Surprisingly, raising [Ca$^{2+}$]o did not increase this fraction, as indicated by the amplitude of the difference current between the first and second current trace in Rb$^+$-Ringer. An explanation for this
behavior could be that Ca\(^{2+}\) is able to leave its binding site even if the channel is closed. The latter possibility is supported by the experiments with an increase in \([\text{Ca}^{2+}]_i\), to \(10^{-5}\) M, which accelerates inactivation and increases the difference current to \(>50\%\).

Several lines of evidence are presented in this paper which indicate that Ca\(^{2+}\) and Ba\(^{2+}\) are able to enter open K\(^+\) channels from the outside. If the channel closes, Ca\(^{2+}\) and Ba\(^{2+}\) become trapped inside the channel. Ba\(^{2+}\) remains trapped, whereas Ca\(^{2+}\) is thought to cross the membrane to the inside during the interval between test pulses. Ba\(^{2+}\) trapping has been described previously for the high conductance Ca\(^{2+}\)-activated K\(^+\) channel from rabbit or rat muscle incorporated into planar lipid bilayers (Vergara and Latorre, 1983; Miller, 1987; Miller et al., 1987; Neyton and Miller, 1988a). The existence of a Ba\(^{2+}\)-trapping mechanism, as well as the previously described sensitivity to charybdotoxin of the Ca\(^{2+}\)-activated "maxi"-K\(^+\) channel and the lymphocyte K\(^+\) channel (Miller et al., 1985; Sands et al., 1988), implies structural similarities between these two channel types and raises the question of whether the K\(^+\) channel in T lymphocytes might be Ca\(^{2+}\) activated. Our experiments with high internal Ca\(^{2+}\) of up to 2 mM show no apparent activation of this channel; instead, raising \([\text{Ca}^{2+}]_i\), speeds up inactivation. One conclusion from our experiments on Ba\(^{2+}\) trapping is that divalent ions in the internal or external solution must wait for the channel to open before entering the pore. If the channel closes, Ba\(^{2+}\) is trapped inside the channel and remains trapped even in the absence of extracellular and intracellular Ba\(^{2+}\). From the sidedness of the Ba\(^{2+}\) action, one has to conclude that both the Ca\(^{2+}\)-activated K\(^+\) channel and the voltage-gated K\(^+\) channel in T lymphocytes have a gating mechanism at the external side that can shield the inside of the channel from externally applied divalent ions, as well as a gating mechanism at the cytoplasmic side of the membrane that shields the inside of the channel from internal blockers (cf. Armstrong, 1971).

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