Voltage-dependent Block of Cardiac Inward-Rectifying Potassium Current by Monovalent Cations

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ABSTRACT The inward-rectifying K⁺ current (IK₁) in cat ventricular myocytes, like inward-rectifying K⁺ currents in many other preparations, exhibited a negative slope conductance region at hyperpolarized membrane potentials that was time-dependent. This was evident as an inactivation of inward current elicited by hyperpolarizing voltage-clamp pulses resulting in a negative slope region of the steady-state current-voltage relationship at potentials negative to -140 mV. Removing extracellular Na⁺ prevented the development of the negative slope in this voltage region, suggesting that Na⁺ can block IK₁ channels in a time- and voltage-dependent manner. The time and voltage dependence of Cs⁺-induced block of IK₁ was also examined. Cs⁺ blocked inward current in a manner similar to that of Na⁺, but the former was much more potent. The fraction of current blocked by Cs⁺ in the presence of Na⁺ was reduced in a time- and voltage-dependent manner, which suggested that these blocking ions compete for a common or at least similar site of action. In the absence of Na⁺, inactivation of IK₁ could also be induced by both Cs⁺ and Li⁺. However, Li⁺ was less potent than Na⁺ in this respect. Calculation of the voltage sensitivity of current block by each of these ions suggests that the mechanism of block by each is similar.

INTRODUCTION

Inward-rectifying K⁺ currents have been identified and studied in many different preparations including frog skeletal muscle (Katz, 1949; Adrian et al., 1970; Almers, 1972a, b; Gay and Stanfield, 1977; Hestrin, 1981; Leech and Stanfield, 1981; Schwarz et al., 1981; Standen and Stanfield, 1978, 1979), starfish egg cells (Hagiwara and Takahashi, 1974; Hagiwara and Yoshii, 1979; Hagiwara et al., 1976, 1978; and Ciani et al., 1980), and tunicate egg cells (Miyazaki et al., 1974; Ohmori, 1978, 1980; Fukushima, 1982). The fact that the currents in these preparations are all very similar suggests that the nature of the channel is well conserved. Studies of the inward-rectifying background current in cardiac ventricular tissue (IK₁) also are consistent with this observation (Noble, 1965; Kameyama et al., 1983; Sakmann and Trube, 1984a, b; Kurachi, 1985; Payet et al., 1985; Josephson and Brown, 1986;

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Biermans et al., 1987; Tourneur et al., 1987; Harvey and Ten Eick, 1988a, b). One of the characteristics of the inward \( K^+ \) current in heart as well as skeletal muscle and tunicate egg cells is a time- and voltage-dependent decline after activation, which becomes greater at more negative membrane potentials. This results in a negative slope conductance region in the steady-state current-voltage relationship.

In frog skeletal muscle the inactivation of inward \( K^+ \) current has been attributed to both a time-dependent change in conductance caused by depletion of \( K^+ \) from extracellular spaces and a time- and voltage-dependent change in permeability (Adrian et al., 1970; Almers, 1972a, b). Depletion alone does not account for the crossover of steady-state current that results in the negative slope conductance region at hyperpolarized potentials (e.g., \( < -140 \) mV). On the other hand, a time- and voltage-dependent block of the ionic current by \( Na^+ \) appears to underlie at least part of this phenomenon because, in tunicate egg cells, removal of extracellular \( Na^+ \) resulted in a linearization of the steady-state I-V relationship (Ohmori, 1978). In addition, analysis of single-channel activity in this preparation has shown that the presence of a channel blocker such as \( Na^+ \) is necessary to even see current fluctuations resulting from channel closing (Ohmori, 1980). In skeletal muscle, it has also been shown that inactivation is at least partially due to a time- and voltage-dependent block of the inward current by extracellular \( Na^+ \) (Standen and Stanfield, 1979). More recently, Biermans et al. (1987) have demonstrated a \( Na^+ \) dependence for the decay of \( I_{K1} \) in isolated guinea pig ventricular myocytes.

In ventricular myocytes isolated from cat hearts, depletion of \( K^+ \) from extracellular spaces can be demonstrated (Harvey and Ten Eick, 1988b), however, it cannot account for the inactivation-like process that produces the negative slope conductance region of the steady-state I-V relationship (Adrian et al., 1970; Almers, 1972b; Harvey and Ten Eick, 1988b). An observation that is consistent with results obtained in guinea pig ventricular myocytes (Biermans et al., 1987) is that the inactivation of \( I_{K1} \) in cat ventricular cells is also sensitive to changes in extracellular \( Na^+ \) concentration (Harvey and Ten Eick, 1988b). This paper describes the time- and voltage-dependent block of the whole-cell inward cardiac \( K^+ \) current by \( Na^+ \), \( Cs^+ \), and \( Li^+ \). The voltage dependence of the block of \( I_{K1} \) by these monovalent cations as well as the interaction between \( Na^+ \) and \( Cs^+ \) suggests that they are acting at a common binding site within the channel. This is consistent with the view of \( K^+ \) channels as multi-ion pores (Hille and Schwarz, 1978; Ciani et al., 1980) and further supports the idea that \( I_{K1} \) is functionally similar to the inward-rectifying \( K^+ \) currents found in other preparations. A preliminary report of this work has appeared in abstract form (Harvey and Ten Eick, 1988a).

**METHODS**

The method used to isolate cat ventricular myocytes is a modification of that originally described by Silver et al. (1983). Whole-cell membrane current was measured using the patch-clamp technique described by Hamill et al. (1981). Specific details concerning these methods have been described previously (Harvey and Ten Eick, 1988b; 1989).

**Data Analysis**

All currents were measured relative to the zero-current level. Peak current was measured as the maximum inward current during a test pulse. Steady-state current was defined operation-
ally as the current level measured at the end of a 75-ms test pulse. The current taken at the end of test pulses longer than 75 ms did not alter the steady-state I-V relationship.

Slope conductance of the inward-rectifying current was determined for the linear portion of the I-V relationship at membrane potentials negative to the reversal potential. A least-squares fitting routine was used to fit these data points and determine the slope.

Solutions

The extracellular solution used during control conditions consisted of (in millimolar): 140 NaCl, 5.4 KCl, 2.5 CaCl₂, 0.5 MgCl₂, 5.0 HEPES, and 5.5 glucose. For the experiments in which the concentration of extracellular Na⁺ was varied between 0 and 125 mM, K⁺ was maintained at 5.0 mM and the other constituents were the same as in the control solution; osmolarity was balanced with tetramethylammonium (TMA) chloride. In some experiments extracellular NaCl was replaced with equimolar concentrations of LiCl. The pH of most external solutions was adjusted to the desired level using NaOH. Unless otherwise indicated, the external pH was 7.4. Using NaOH to raise the pH to this level added ~2.5 mM Na⁺ to the solutions. In experiments with varying [Na⁺]o this additional Na⁺ is included in the reported concentration. Also, some experiments used solutions in which the pH had been adjusted using TMA-OH. The intracellular solution contained (in millimolar): 130 K-glutamate, 10.0 KCl, 1.0 MgCl₂, 1.0 ATP-K₃, 5.0 EGTA, and 5.0 HEPES; pH 7.2. The pH of the internal solution was adjusted using KOH. Control conditions are defined as those in which both control extracellular and intracellular solutions were used. Temperatures were maintained at 32 ± 1°C using a Peltier thermo-electric device.

RESULTS

The results of previously published reports (Biermans et al., 1987; Harvey and Ten Eick, 1988a) indicate that extracellular Na⁺ can play some role in the inactivation of Iₖ at extremely hyperpolarized membrane potentials. This is consistent with the characteristics of the inward-rectifying K⁺ currents described for frog skeletal muscle and tunicate egg cells (Ohmori, 1978, 1980; Standen and Stanfield, 1979; Fukushima, 1982). An example of the Na⁺ dependence of Iₖ is illustrated in Fig. 1. The current traces in A were obtained using the voltage-clamp protocol used for most of these experiments. The membrane was held at −40 mV and Iₖ was activated with 75-ms hyperpolarizing test pulses. In this figure current was elicited by test pulses to −170 mV under conditions where the extracellular Na⁺ concentration was varied between 0.0 and 125.0 mM (using TMA as the replacement ion). It is apparent that the total amount of inactivation decreased as Na⁺ was removed. This is reflected as a decrease in the steepness of the negative slope region of the steady-state I-V relation obtained with the same preparation (Fig. 1 B).

Sodium Dependence of Inactivation

The amount of current that inactivates in the presence of various extracellular Na⁺ concentrations was estimated from the difference between the peak and steady-state current elicited during a given test pulse (see inset, Fig. 2 A). Because maximal Iₖ conductance changes when Na⁺ is varied (Harvey and Ten Eick, 1989), the current measurements were normalized with respect to the peak slope conductance at each Na⁺ concentration. This is reasonable because activation of Iₖ was found to be Na⁺ independent (Harvey and Ten Eick, 1989). As extracellular Na⁺ was reduced, the current-voltage relationship of the normalized inactivating current indicates that, as
expected, if Na<sub>e</sub> were responsible, the relative amount of current inactivated was reduced (Fig. 2 A).

The Na<sup>+</sup> dependence of the normalized inactivating current was characterized by plotting the inactivating current measured at −170 mV against Na<sub>e</sub> concentration (Fig. 2 B). It appears that under these conditions, extracellular Na<sup>+</sup> is responsible for ~60% of I<sub>K1</sub> inactivation at −170 mV, and the Na<sup>+</sup>-dependent inactivation process was saturated at the extracellular Na<sup>+</sup> concentrations used during control conditions (i.e., 145 mM Na<sup>+</sup>).

**Cesium-induced Inactivation**

The monovalent ion cesium can effectively block most K<sup>+</sup> currents including I<sub>K1</sub> in ventricular myocytes (Kameyama et al., 1983; Sakmann and Trube, 1984a; Tourner et al., 1987; Harvey and Ten Eick, 1988b). In starfish egg cells the block of inward-rectifying K<sup>+</sup> current by Cs<sup>+</sup> exhibits a voltage and time dependence (Hagiwara et al., 1976), which in many respects resembles the Na<sup>+</sup> block of I<sub>K1</sub>. The voltage and time dependence of Cs<sup>+</sup> block of I<sub>K1</sub> in cat ventricular myocytes is illustrated in Fig. 3. Fig. 3 A shows the fraction of current remaining at the end of a 75-ms test pulse after exposure to various concentrations of Cs<sup>+</sup> at membrane potentials between −100 and −180 mV. At any potential, the amount of current blocked by Cs<sup>+</sup> increased as the concentration of blocking ion was increased, and at any concentration of Cs<sup>+</sup> the fraction of current remaining in the presence of Cs<sup>+</sup> decreased as the membrane potential was made more negative. Fig. 3 B shows the
fraction of current remaining after exposure to either 0.01 or 0.1 mM Cs⁺ at various time points during a test pulse to -140 mV. This indicates that the time course of Cs⁺ block of Iₖ was faster at higher concentrations.

The observation that Cs⁺, like Na⁺, acts in a time- and voltage-dependent manner leads to the question of whether the effects of Cs⁺ on Iₖ might be due to an effect involving the same site at which Na⁺ induces its Iₖ blocking or inactivating properties, but perhaps having a much higher affinity for the site. To examine this possibility more closely, low concentrations of Cs⁺ were used to improve the resolution of the time dependence of Cs⁺-induced block. Fig. 4 A shows currents obtained before and after 0.01 mM Cs⁺ was added to the extracellular solution. The effect of Cs⁺ was most noticeable at more negative membrane potentials where the rate and extent of inactivation was enhanced. This is seen more clearly in the steady-state I-V relationship depicted in Fig. 4 B. Cs⁺ only reduced inward current at potentials negative to -120 mV. Fig. 4 C shows the fraction of inward K⁺ current remaining in

FIGURE 2. Sodium dependence of inactivation. (A) Current-voltage relationship of normalized inactivating current (iₙ₇₅) obtained when [Na⁺]ₒ was varied (extracellular Na⁺ replaced with TMA). (Inset) The amount of current which inactivated during a test pulse (iₙ₇₅) was calculated as the difference between peak (iₚₑᵃᵏ) and steady-state (iₙ) current. These values were normalized for Nₐ-Dependent changes in conductance by multiplying iₙ₇₅ by the ratio of the slope conductance calculated when [Na⁺]ₒ was maximal (gₑₐ₇₅) relative to the slope conductance calculated when [Na⁺]ₒ was reduced (gₑₐ₇₅). Slope conductance was determined by measuring the slope of the peak I-V relationship at potentials negative to the reversal potential by fitting data points with a linear regression. (B) Sodium dependence of Iₖ inactivation. iₙ₇₅ was calculated from experiments in four different cells exposed to various [Na⁺]ₒ. The value of iₙ₇₅ at -170 mV was used as an indication of the degree of Iₖ inactivation. Normalized values from four cells are plotted as a function of [Na⁺]ₒ. Various symbols represent data from different cells. Extracellular Na⁺ was replaced with TMA.
the presence of 0.01 mM Cs⁺ during the course of 75-ms test pulses to voltages between -140 and -180 mV. It is evident that the degree of steady-state block at the holding potential (-40 mV) was very small and that the block of current at more negative potentials developed with time. However, at potentials more negative than -160 mV, the amount of current remaining in the presence of Cs⁺ began to increase again after 5–10 ms. This feature of Cs⁺ block appeared to correlate temporally with the voltage-dependent block of IₓK by Na⁺.

The apparent decline of Cs⁺ block can be explained if Cs⁺ is acting at the same site within the channel as Na⁺. Therefore, this most probably reflects a difference in

![Figure 3](https://example.com/figure3)

**Figure 3.** Voltage and time dependence of current block by Cs⁺. (A) Fraction of steady-state current measured in the presence of 0.01, 0.1, 0.5, and 1.0 mM Cs⁺ (Iₓw/LH) relative to the steady-state current measured in the absence of Cs⁺ (Iₓw/LH≈0) plotted as a function of membrane potential (Vₓ). (B) Fraction of current blocked by 0.01 and 0.1 mM Cs⁺ at various time points during a 75-ms test pulse to -140 mV. Control intracellular solution; control extracellular solution with or without Cs⁺. Data are from one representative cell.

delays of development of block by Na⁺ and Cs⁺ at a common site. At membrane potentials that normally exhibit block of IₓK by Na⁺ (< -140 mV), there may be an interaction or competition between the two ions, which results in a decrease in the relative amount of current blocked by Cs⁺. To test this possibility, the Cs⁺-induced block was examined under Na⁺-free conditions. In Fig. 5 A a family of currents elicited by test pulses between -40 and -180 mV in the absence of Na⁺ are shown. B shows the currents in the same cell when 0.01 mM Cs⁺ was added to the external solution. It is evident that Cs⁺ had a more pronounced effect on the currents elicited at more negative potentials. However, this concentration of Cs⁺ was not able to completely eliminate inward K⁺ current, even at -180 mV. It is interesting to note
that the time and voltage dependence of the \( \text{Cs}^+ \) block closely imitated the time- and voltage-dependent inactivation of \( I_{K1} \) seen under control conditions (i.e., 145 mM \( \text{Na}^+ \)). This is more clearly visualized in the steady-state current-voltage relationships of the above currents (Fig. 5 B). In the absence of \( \text{Na}_a \) there was no crossover of the steady-state currents at potentials negative to \(-140 \text{ mV}\) as is evident because of the lack of a negative slope region in the I-V relationship. However, the addition of just 0.01 mM \( \text{Cs}^+ \) induced a voltage-dependent inactivation, and produced a steady-state

![Figure 4](https://jgp.rupress.org/)

**Figure 4.** Time dependence of \( I_{K1} \) block by \( \text{Cs}^+ \) in the presence of extracellular \( \text{Na}^+ \). (A) Family of currents obtained before (a) and after (b) exposure to 0.01 mM \( \text{Cs}^+ \). Calibration: 3 nA; 10 ms. (B) Current-voltage relationships of steady-state currents shown in previous panels. (C) Fraction of current remaining after exposure to 0.01 mM \( \text{Cs}^+ \) \( \left( I_{\text{WITH} \text{Cs}^+} / I_{\text{WITHOUT} \text{Cs}^+} \right) \) plotted as a function of time during a 75-ms test pulse (TP) to membrane potentials between \(-140\) and \(-180 \text{ mV}\). Control extracellular solution with or without \( \text{Cs}^+ \). The data points were fit to an exponential function by a sum of least-squares curve-fitting program. The time course of block was fit by one exponential at potentials positive to \(-160 \text{ mV}\) and two exponentials at the more negative membrane potentials.

I-V curve, which suggests that an inactivation similar to that seen in the presence of \( \text{Na}^+ \) can be induced by \( \text{Cs}^+ \) in the absence of \( \text{Na}^+ \).

The idea that \( \text{Na}^+ \) and \( \text{Cs}^+ \) might compete for a common site involved with the inactivation process was examined further. If the two-ion species do compete, the relative amount of current remaining in the presence of \( \text{Cs}^+ \), but in the absence of \( \text{Na}^+ \), should reach a minimum and not increase again at the more hyperpolarized membrane potentials. When the time course of \( \text{Cs}^+ \) block was examined in the absence of \( \text{Na}_a \) (Fig. 5 C), the time-dependent decline of \( \text{Cs}^+ \)-sensitive current, seen when \( \text{Na}^+ \) was present, disappeared (compare Figs. 4 and 5). This is consistent with
the hypothesis that Cs⁺-induced block of IKᵢ is at least partly due to an interaction of the ion with the same site involved in Na⁺-induced inactivation.

**Lithium Dependence of Inactivation**

When Li⁺ was used as a Na⁺ substitute, it too appeared to support inactivation. Fig. 6A shows current traces obtained when Na⁺ had been replaced with either TMA or Li⁺. It was observed that when Na⁺ was replaced with Li⁺, as opposed to TMA,

![Current traces](image)

**Figure 5.** Cesium-induced inactivation of IKᵢ in the absence of extracellular Na⁺. (A) Currents elicited during test pulses (TP) between -40 and -180 mV in 10 mV steps. (a) Currents elicited in the absence of Na⁺, (b) currents elicited in the presence of 0.01 mM Cs⁺ but in the presence of 0.01 mM Na⁺. (B) Current voltage relationships of steady-state current obtained from experiments shown in previous panels. (C) Time dependence of IKᵢ block by Cs⁺ in the absence of extracellular Na⁺. Fraction of current remaining after exposure to 0.01 mM Cs⁺ (Iᵢₖₛₑₚ/Iᵢₖₚₑₚ) plotted as a function of time during a 75-ms test pulse to membrane potentials between -140 and -180 mV. Extracellular Na⁺ was replaced with TMA. The data points were fit to an exponential function by a sum-of-least-squares curve-fitting program. The time course of block was fit by a single exponential at all potentials.

there was still a crossover of the steady-state currents at more negative potentials. This is seen more clearly in the steady-state I-V relationships shown in Fig. 6B. This is consistent with the single-channel data of Ohmori (1982) who showed that Li⁺ can block the inward-rectifying K⁺ channels of tunicate egg cells. In addition, the time and voltage dependence of Li⁺ block of IKᵢ is very much like that described above for Na⁺ and Cs⁺. The amount of inactivation produced by Na⁺ is greater
than that caused by Li$^+$. In fact, the degree of inactivation (at $-170$ mV) induced by 125 mM Li$^+$ is similar to the level of inactivation produced by $\sim 50$ mM Na$^+$.

**Voltage-dependent Block of $I_{KI}$**

It appears that each monovalent cation may induce inactivation of $I_{KI}$ in a similar manner: by inducing a voltage-dependent block of $I_{KI}$. To determine whether these blockers might be acting at a common site, the fraction of the voltage drop ($V_M$) across the membrane which is sensed by the inactivation mechanism ($\delta$) was calculated using the equation:

$$\ln \left( \frac{I}{I_B} - 1 \right) = \ln \left( \frac{[B]_o [K_f]/[K_{-1}]}{1} \right) + \frac{\delta z F V_M}{RT}$$

where $I$ is the steady-state current in the absence of a blocker; $I_B$ is the steady-state current in the presence of the blocker (B); $K_f$ and $K_{-1}$ are the forward and reverse rate constants (respectively) for the blocking reaction; and $z$, $F$, $R$, and $T$ have their usual thermodynamic meanings. $\delta$ can be calculated from the slope of the relationship between $\ln \left( \frac{I}{I_B} - 1 \right)$ and $V_M$. In addition, the dissociation constant ($K_a$) for each blocker can be calculated by extrapolating the same relationship back to $V_M = 0$ mV. At this potential $K_a = [B]_o / \ln \left( \frac{I}{I_B} - 1 \right)$. Table I lists the calculated values of $\delta$ and $K_a$ for each ion from representative experiments.

Values of $\delta > 1$ suggest that the inactivation mechanism senses $>100\%$ of the potential across the membrane (Ohmori, 1980; Hille, 1984). The values reported in Table I are similar to those reported by Ohmori (1980) for the block of inward-rectifying potassium channels in tunicate egg cells by Na$^+$ (1.31) and Cs$^+$ (1.46). He suggested that $\delta$ was $> 1$ because the block by these ions was cooperative with other cations, most likely K$^+$. The similarity of the values calculated for the voltage-dependent block of $I_{KI}$ by Na$^+$, Li$^+$, and Cs$^+$ suggests that the monovalents act in a similar...
manner, possibly at the same site in the IKᵢ channel. From the Kᵣ values, the relative potency of these ions in mediating inactivation was determined to be Cs⁺ >> Na⁺ > Li⁺.

DISCUSSION

Inactivation has been demonstrated for almost all noncardiac inward-rectifying K⁺ currents. This includes tunicate egg cells (Ohmori, 1978, 1980; Fukushima, 1982) and frog skeletal muscle (Standen and Stanfield, 1979). In each of these cases at least some portion of the inactivation has been attributed to a time- and voltage-dependent block of the current by Na⁺. In contrast, in starfish egg cells, while some inward-rectifying current inactivates, the degree to which it inactivates is much less than that seen in the other preparations (Hagiwara et al., 1978). However, a pronounced increase in a time- and voltage-dependent inactivation can be produced by exposure to low concentrations of Cs⁺ or Ba²⁺ (Hagiwara et al., 1976, 1978). In tunicate egg cells, where inactivation has been most extensively studied, the presence of Na⁺ as well as other ions could induce the current fluctuations associated with single-channel activity. The other ions included Cs⁺, Li⁺, Sr²⁺, and Ba²⁺ (Ohmori, 1980). These findings have suggested that ions such as these can cause a potential- and time-dependent block of inward-rectifying K⁺ channels and thereby induce inactivation of inward K⁺ current. The results of the present study suggest that inactivation of inward IKᵢ in isolated cat ventricular myocytes may also be mediated by a similar mechanism that can involve Na⁺ as well as other monovalent cations.

Sodium-dependent Inactivation of IKᵢ

Josephson and Brown (1986) have suggested that the inactivation of IKᵢ in isolated rat ventricular myocytes is caused by an intrinsic change in the probability for opening of the inward-rectifying channel. This hypothesis was developed because removal of extracellular Na⁺ neither completely removed the time-dependent decline of the inward current at more negative membrane potentials nor did it eliminate the negative slope region of the steady-state I-V relationship. In cat ventricular myocytes, on the other hand, the effect of removing Na⁺ suggests that this ion at

| Voltage-Dependent Block of IKᵢ | δ   | Kᵣ     |
|-------------------------------|-----|--------|
| Na⁺                           | 1.57| 5.62 x 10⁻³ |
| Li⁺                           | 1.18| 1.40 x 10⁻² |
| Cs⁺                           | 1.34| 1.11 x 10⁻⁴ |

The fraction of the electrical drop across the membrane, which is sensed by the inactivation mechanism (θδ), was calculated using the equation: ln [(I/I₀) - 1] = ln [θδ(Kᵣ/K₀)] + δVᵣF/RT. δ was calculated from the slope of the relationship between ln [(I/I₀) - 1] and Vᵣ. Kᵣ was calculated by extrapolating the same relationship back to Vᵣ = 0. In the case of Na⁺, current in the absence of Na⁺ (I) was normalized to account for the Na⁺ dependence of fᵢᵢ conductance.
least partially mediates the inactivation of $I_{K1}$. The hypothesis that the inactivation process involves a Na$^+$-dependent block also receives support from recently reported findings in guinea pig ventricular cells (Biermans et al., 1987).

In cat ventricular cells Na$^+$-dependent inactivation of $I_{K1}$ accounted for $\sim 60\%$ of the time-dependent decline in $I_{K1}$ from the peak level at $-170$ mV (see Fig. 4). The remainder of the decline appears to be associated with K$^+$ depletion. This is in contrast to the findings in guinea pig ventricular myocytes (Biermans et al., 1987). We have previously reported that under similar experimental conditions, after a 75-ms test pulse to $-170$ mV, a change in $E_K$ capable of accounting for $\sim 40\%$ of the current decline from peak levels can occur as a result of depletion-induced change in driving force and an associated extracellular K$^+$-dependent change in conductance (Harvey and Ten Eick, 1988b). Therefore, the Na$^+$-independent component of $I_{K1}$ inactivation under control conditions appears to be primarily due to depletion of extracellular K$^+$ from the unstirred solution layer in the peri-sarcolemmal space surrounding the myocytes. It is conceivable that some portion of the Na$^+$-independent inactivation was produced by a voltage-dependent block induced by other external cations, such as Ca$^{2+}$. However, in our experiments, when cells were exposed to 0 mM Na$^+$ using TMA as the substitute ion, complete removal of Ca$^{2+}$ did not affect the degree of time-dependent inactivation (data not shown). This differs from the results of Biermans et al. (1987) who found that in the absence of Na$^+$, a fraction of time-dependent decline was eliminated upon removal of Ca$^{2+}$ and Mg$^{2+}$.

**Sodium Dependence of Inactivation and Conductance**

It has been shown (Harvey and Ten Eick, 1989) that removing Na$_o$ reduces the size of the peak inward K$^+$ current. The fact that altering the extracellular Na$^+$ concentration can affect $I_{K1}$ conductance as well as $I_{K1}$ inactivation opens the possibility that, as has been suggested for tunicate egg cells (Fukushima, 1982) and rat ventricular myocytes (Payet et al., 1985), these two responses may be coupled. However, whereas the Na$^+$ dependence of inactivation is believed to be a direct extracellular effect, the results of our previous report (Harvey and Ten Eick, 1989) suggest that the Na$_o$ dependence of $I_{K1}$ conductance is an indirect effect of Na$_o$ mediated through the dependence of intracellular pH on Na$^+$-proton exchange. Therefore, altering intracellular pH independent of extracellular Na$^+$ concentration should permit the Na$^+$-dependent changes in conductance to be separated from the Na$^+$ dependence of the inactivation process.

**Block by Monovalent Cations**

Cat ventricular myocytes exposed to Cs$^+$ exhibited a time- and voltage-dependent block of $I_{K1}$. This was most evident at low concentrations (i.e., 0.01 mM) of the blocking ion. When Na$^+$ was present, the finding of a time-dependent decline in the fraction of the current blocked by Cs$^+$ at potentials negative to $-140$ mV (see Fig. 4) suggests that these two ions compete for a site in the $I_{K1}$ channels involved in the inactivation process. In the absence of extracellular Na$^+$, addition of 0.01 mM Cs$^+$ to the extracellular solution was able to induce an inactivation that resembled that seen with concentrations of Na$^+$ $>100$ mM. This finding suggests that this "inactiva-
tion" site has a higher affinity for Cs⁺ than for Na⁺. Assuming that Li⁺ also acts at this site, the observation that Li⁺ was not able to induce the same degree of inactivation as an equal concentration of Na⁺ suggests that the channel site has a higher affinity for Na⁺ than for Li⁺. Therefore, the relative order for the efficacy of these ions to induce inactivation appears to be Cs⁺ > Na⁺ > Li⁺. This is consistent with the calculated $K_d$ values (see Table I).

The steepness of the voltage dependence of block by all three monovalent cations suggests that they are all acting at a site within the channel and that movement to this site may be coupled to the movement of K⁺ to a second site within the channel (Ciani et al., 1980; Ohmori, 1980). These results are then consistent with the theory that the $I_{K1}$ channel is a multi-ion pore (Hille and Schwarz, 1978).

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