J. Membrane Biol. 122, 33–42 (1991)
0022263191000784

The Journal of
Membrane Biology

Permeance of Cs^+ and Rb^+ through the Inwardly Rectifying K^+ Channel in Guinea Pig Ventricular Myocytes

Raman L. Mitra and Martin Morad
Department of Internal Medicine, Cardiovascular Division, and Department of Physiology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

Summary. Inward currents carried by external Cs, Rb, NH_4 and K through the I_{K1} channel were studied using a whole-cell voltage clamp technique. Cs, NH_4, and Rb currents could be recorded negative to −40 mV following depolarizing prepulses (≥0 mV and 200–1000 msec in duration). The current activation displayed an instantaneous component followed by a monoexponential increase (τ_a) to a peak amplitude. Subsequent inactivation was fit by a single exponential, τ_{ia}. With hyperpolarization, τ_a and τ_{ia} decreased *e*-fold per 36 and 25 mV, respectively. In Ca-free external solutions (pipette [Mg] ≈ 0.3 mM), inactivation was absent, consistent with the hypothesis that inactivation represents time- and voltage-dependent block of Cs, NH_4, and Rb currents by external Ca. The inactivation and degree of steady-state block was greatest when Cs was the charge carrier, followed by NH_4, and then Rb. K currents, however, did not inactivate in the presence of Ca. Na and Li did not carry any significant current within the resolution of our recordings. Comparison of *peak* inward current ratios (I_x/I_K) as an index of permeability revealed a higher permeance of Cs (0.15), NH_4 (0.30), and Rb (0.51) relative to K (1.0) than that obtained by comparing the *steady-state* current ratios (Cs : NH_4 : Rb : K ≈ 0.01 : 0.06 : 0.21 : 1.0). At any given potential, τ_a was smaller the more permeant the cation. In the absence of depolarizing prepulses, the amplitude of τ_a was reduced. Divalent-free solutions did not significantly affect activation in the presence of 0.3 mM pipette [Mg]. When pipette [Mg] was buffered to ≈ 50 μM, however, removal of external Ca and Mg lead to a four- to fivefold increase in Cs currents and loss of both time-dependent activation and inactivation (reversible upon repletion of external Ca).

These results suggest that (*i*) permeability ratios for I_{K1} should account for differences in the degree to which monovalent currents are blocked by extracellular Ca and (*ii*) extracellular or intracellular divalent cations contribute to the slow phase of activation which may represent either (a) the actual rate of Mg or Ca extrusion from the channel into the cell, a process which may be enhanced by repulsive interaction with the incoming permeant monovalent cation or (b) an intrinsic gating process that is strongly modulated by the permeant monovalent ion and divalent cations.

Key Words heart · inward rectification · potassium channel · selectivity · gating · Ca · Mg

Introduction

The possibility that the gating particles in Na channels might be Ca ions was suggested by Frankenhaeuser and Hodgkin (1957). Although now it is generally felt that the gating charges of the Na channel are intrinsic to the protein (reviewed by Armstrong, 1981), evidence has accumulated that divalent cations, particularly Ca and Mg, act as either the gating particle or as a modulator of the gating system in both inwardly and outwardly rectifying K channels (Armstrong & Lopez-Barneo, 1987; Biermans, Vereecke & Carmeliet, 1987; Matsuda, Saigusa & Irisawa, 1987; Mitra & Morad, 1987; Vandenberg, 1987; Grissmer & Cahalan, 1989; *cf.* Silver & DeCoursey, 1990). It is well known that micromolar concentrations of extracellular Ba cause time- and voltage-dependent block of I_{K1} in oocytes and skeletal muscle (Hagiwara et al., 1978; Standen & Stanfield, 1978). Hagiwara et al. (1978) also demonstrated that millimolar concentrations of Ca or Sr are required to cause a similar block, suggesting that Ca or Sr bind to the channel with a lower affinity than Ba.

The purpose of this study was to examine the possibility that extracellular Ca causes inactivation and block of currents carried by the less permeant monovalent alkali cations, Cs, NH_4, and Rb, in a manner similar to Ba block of K currents. The effect of altering the intracellular [Mg] on the slow phase of activation (Kurachi, 1985; Mitra, Morad & Tourneur, 1985; Tourneur et al., 1987) was also examined in the presence and absence of external divalent cations. While recent work suggests that the time-dependent activation of I_{K1} represents an intrinsic gating process (Ishihara et al., 1989; Silver & DeCoursey, 1990), the findings presented here suggest that interaction of the permeant ion with divalent

cations on either side of the membrane is responsible for the observed gating behavior. A similar conclusion has been reported for an outwardly rectifying K channel in human T lymphocytes (Grissmer & Cahalan, 1989).

Materials and Methods

Guinea pig ventricular myocytes were enzymatically isolated (Mitra & Morad, 1985), voltage clamped and internally dialyzed using a single electrode whole-cell voltage-clamp technique (Hamill et al., 1981). WPI Kwik-Fil tubing was used to fabricate pipettes with tips ranging from 2–5 μm and having resistances of 0.5–5 MΩ. Tips were not fire polished. A Dagan 8900 amplifier with a 100-MΩ feedback resistor in the headstage served as the voltage follower and current-injecting source. The series resistance (80–85%) was effectively compensated using a feedback circuit. The currents were displayed on a Textronics D11 storage oscilloscope, recorded on Polaroid film and were subsequently digitized and analyzed. Exponential curves were fit using a least-squares program. Current data were not leak subtracted. The first 1 msec of the current tracing which contained the capacitive transient was deleted (unless otherwise specified) since the time course of the currents being studied was much slower. The external solutions were as follows (in mM): sucrose 180, 120 or 0 when *M*Cl 30, 60, or 120, respectively; $CaCl_2$ 2 or 5; HEPES 10; glucose 10; $MgCl_2$ 0.5; pH = 7.40 with *M*OH, where $M = Cs^+$, Rb^+, K^+, NH_4^+, Na^+, or Li^+. The internal pipette solution contained (in mM): N-methyl-D glucamine 120, HCl 60, gluconic acid 60, MgATP 2, EGTA 10, HEPES 10, pH = 7.40 with N-me-D-glucamine. Addition or deletion of specific ions deviating from the standard solutions are noted in the figure legends. N-methyl-D-glucammonium was the only cation used in the internal pipette solution to minimize possible interactive effects that permeant internal cations might have with channel binding sites. All experiments were done at 23–25°C. Pipette free [Mg] was calculated using a computer program (kindly provided by H. Droogmans). Stability constants of ATP-*X*, citrate-*X*, and EGTA-*X* (*X* = Ca or Mg) were from Martell and Smith (1977). In this paper reference to intracellular Mg^{2+} concentration ($[Mg^{2+}]_i$) assumes equilibration of pipette and intracellular $[Mg^{2+}]$, which may not strictly be true.

Valency superscripts will be omitted in the text and should be assumed to be +1 for alkali and +2 for alkaline earth and transition metals.

Results

Cs Current through the Inward Rectifier

When Cs, Ca and Mg were the only cations in the extracellular solution, a slowly inactivating inward tail current was noted at −80 mV following a clamp pulse to +80 mV (Fig. 1*a*). The current was characterized by an instantaneous activation followed by a slower time-dependent increase. Subsequent inactivation decreased the current to a steady-state level (holding current). The holding and peak inward currents were enhanced about twofold when $[Cs]_o$ was increased from 60 to 120 mM (downward arrows). Replacing Cs by Tris suppressed the tail current (Fig. 1*b*). These effects were completely reversible and suggested that the tail current was carried by Cs.

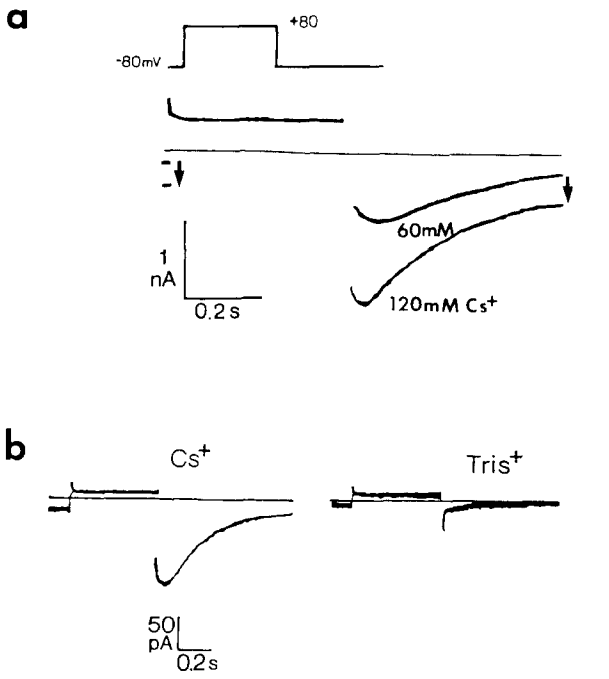

Fig. 1. Inward Cs tail currents following a depolarizing pulse. (*a*) Inward holding and peak inward tail current at −80 mV increase (downward arrows) as $[Cs]_o$ is raised from 60 to 120 mM. $[Ca]_o$ = 2 mM (cell 8-21-142). (*b*) Substitution of Cs (30 mM) with equimolar $Tris^+$ (in another cell) suppresses the tail current. $[Ca]_o$ = 5 mM (cell 3-15-37). Note the absence of change in the outward current at +80 mV indicating that the observed effects are not due to changes in leak current

Attempts were then made to identify the channel through which Cs was permeant. Figure 2*a* compares the effect of two different conditioning pulses (0 and +100 mV) on the magnitude of Cs tail currents activated at −80 mV in the presence of 15 μM TTX. The conditioning clamp to +100 mV was associated with the larger inward tail current. Addition of 5 mM Co to the external solution completely blocked the inward Ca current at 0 mV, but had little or no effect on the Cs tail currents. These experiments indicate that the inward Cs tail current was not carried by either TTX-sensitive Na channels or Ca channels. Figure 2*b* shows that the inward Cs tail current was blocked by 50 μM Ba added to the external solution. Higher concentrations of Sr (0.5 mM) were required to achieve the same degree of block (Fig. 2*c*). These effects were reversible upon washing out the divalent ion. Since Ba and Sr at these concentrations are

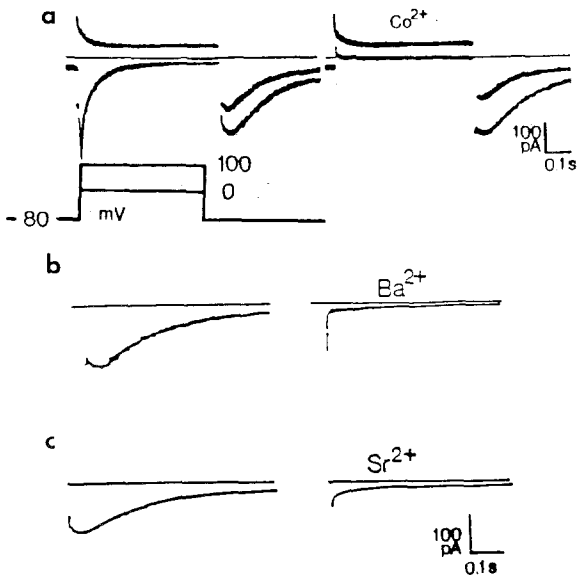

Fig. 2. Cs current is through the inwardly rectifying K channel and enhanced by depolarization. (*a*) In the left panel, Cs (30 mM) current is larger following the depolarizing pulse to +100 mV (15 μM TTX present). Ca current is activated with a pulse to 0 mV followed by the smaller Cs tail current. $[Ca]_o$ = 5 mM. In the right panel, addition of 5 mM Co completely blocks Ca current but does not affect the inward Cs currents (cell 1-14-3). (*b*) and (*c*) Fifty μM Ba (cell 3-15-37) and 500 μM Sr (cell 3-20-45) block the inward Cs (30 mM) current. Same voltage protocol as *a*, but only tail currents following a depolarizing pulse to +100 mV are shown. $[Ca]_o$ = 5 mM

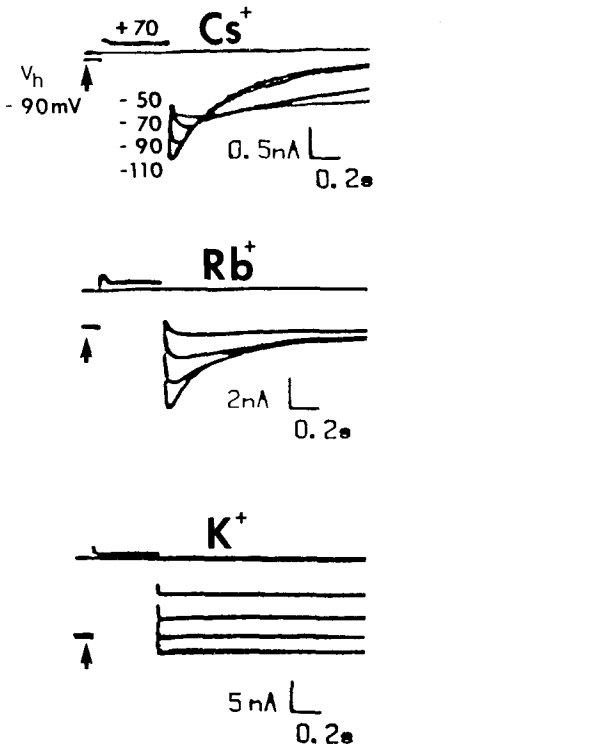

Fig. 3. Comparison of Cs, Rb, and K (120 mM) currents at −50, −70, −90 and −110 mV following a depolarizing clamp pulse to +70 mV in the same cell (cell 8-21-143). Holding potential (H_p) = −90 mV. Note the increase of holding current (arrow) on going from Cs to Rb to K and the different current calibration. Return to Cs after the K solution resulted in identical currents as the control Cs solution (*not shown*). $[Ca]_o$ = 2 mM

known to block I_{K1} (Hagiwara et al., 1978; Standen & Stanfield, 1978; Tourneur et al., 1987), the results of Fig. 2 indicate that Cs is permeant through the inwardly rectifying K channel. The activation of the Cs current with hyperpolarization also suggests that it is carried by I_{K1} channels, since no other cardiac channel is known to activate at such negative potentials.

Cs Currents Inactivate Due to Ca Block

The results thus far suggest that the conductance of the channel through which Cs was permeant increased with depolarization. These findings were somewhat surprising since the conductance of I_{K1} decreases with depolarization (Katz, 1949). To explore the possibility that inactivation represented an ion-dependent process, Cs was replaced by Rb, and then K, in the same cell (Fig. 3). The top panel shows Cs currents activated at test potentials of −50, −70, −90 and −110 mV following a depolarizing prepulse. Both activation and inactivation became more rapid with hyperpolarization. Isomolar substitution with Rb (middle panel) resulted in larger currents which displayed faster activation but a lesser degree of inactivation; K currents displayed no inactivation, activated more rapidly and were about 10-fold greater than the Cs currents (bottom panel). These results suggest that time- and voltage-dependent inactivation is not an intrinsic property of the channel but is strongly dependent on the permeant ion.

To examine the possibility that Ca ions were causing inactivation of Cs currents, the effect of changing extracellular Ca was examined. Figure 4*a* demonstrates that removal of external Ca (and Mg) completely suppressed inactivation while not significantly affecting the time-dependent phase of activation. The results were completely reversible upon return to the Ca-containing solution (n = 6). Increasing $[Ca]_o$ from 5 to 10 mM (Fig. 4*b*) reduced both the inward holding current and the peak inward Cs current by about 0.4 (mean = 0.41 ± 0.04, n = 5). Note that no change occurred in the outward current at +80 mV in either Fig. 4*a* or *b*, indicating that the changes in holding current at −80 mV were not due to induction of a nonspecific linear leak conductance. Similar results were observed with Rb and

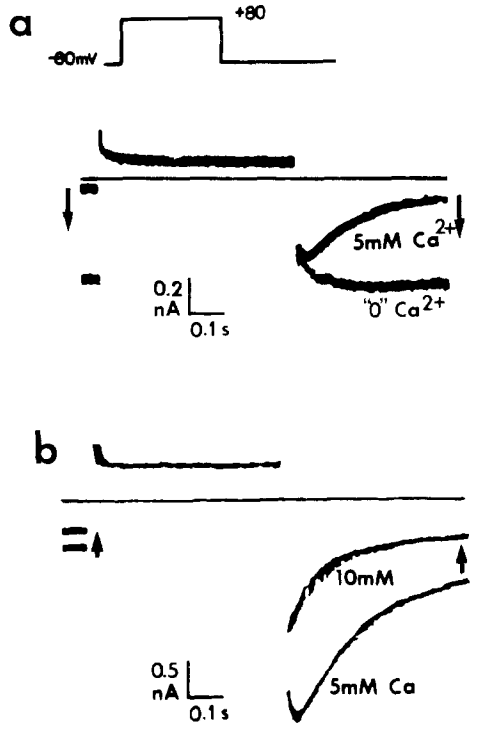

Fig. 4. External Ca and Mg responsible for inactivation. (*a*) Removal of external Ca and Mg eliminates inactivation. Note the increase in the inward Cs (30 mM) holding current but lack of effect on the outward current at +80, indicating that the increase in inward current is not due to an increase in nonspecific leak conductance $[Ca]_o$ = 5 mM (cell 3-4-23). (*b*) Increasing external Ca from 5 to 10 mM decreases the Cs (60 mM) inward holding current and the tail current but does not affect the outward current (cell 3-20-46)

NH_4 (*not shown*). These results suggested that inactivation was due to time- and voltage-dependent Ca block, analogous to time- and voltage-dependent block of K currents by Ba (Hagiwara et al., 1978; Standen & Stanfield, 1978). The depolarizing conditioning pulses would remove Ca block of the channel, thus permitting activation of current on repolarization, following which time- and voltage-dependent Ca block recurs.

Mg in the absence of external Ca also caused inactivation of Cs currents to a lesser degree. We did not study the effects of external Mg in detail; however, the presence of 0.5 mM Mg in the external solution improved the long-term stability of cells in Ca-containing solutions.

Voltage Dependence and Kinetics of Cs Current

In Fig. 2, the peak inward Cs current at −80 mV was much larger following the conditioning pulse to +100 mV compared to 0 mV, consistent with voltage-dependent block by Ca. Since there was also a time-dependent component to the Ca block, the following questions were examined: (*i*) Does the duration of the depolarizing conditioning pulse affect the degree of unblock at a given conditioning potential? (*ii*) Is τ_{ia} voltage dependent? Figure 5*a* shows that the amplitude of peak inward Cs current increases as the duration of the conditioning pulse (+70 mV) increases. No significant increase in Cs tail current was noted when the duration of the conditioning pulse exceeded 1 sec, suggesting that the process of unblock had reached its steady-state value. Figure 5*b* is a semilogarithmic plot in which the ordinate is the difference between the peak inward Cs current elicited after a 2-sec conditioning pulse and that following pulses of shorter duration, while the abscissa is the duration of the conditioning pulse. The increase of the Cs tail current envelope was fit by a single exponential (τ = 520 msec). With conditioning pulses between 60 to 80 mV, the average time constant for this process was 510 ± 50 msec in the presence of 5 mM external [Ca] (n = 9). Thus, the rate of Ca unblock is quite slow even at very positive potentials.

Kinetic analysis of the Cs currents revealed that at each potential, the current could be fitted accurately with the sum of two exponentials, corresponding to activation and inactivation. This is illustrated in Fig. 6*a* where current records (dots) are shown superimposed on exponential curves (solid lines). The equations for the curves are given in the figure legend. Both τ_a and τ_{ia} decreased with hyperpolarization. From the slopes of the linear regression analysis of $\ln(\tau_a)$ and $\ln(\tau_{ia})$ as a function of membrane potential (Fig. 6*b*, n = 5), τ_a and τ_{ia} were found to decrease e-fold per 36 and 25 mV, respectively, over the voltage range studied. The voltage dependence of $\ln(\tau_{ia})$ was nonlinear negative to −100 mV and τ_{ia} reached a minimum limiting value of 20 to 30 msec negative to −120 mV. The voltage dependence of τ_{ia} is consistent with voltage-dependent block by extracellular Ca.

Effects of Holding Potential on Activation

To further examine the role of the depolarizing conditioning pulses on activation and inactivation, inward Cs currents were activated on hyperpolarization from negative holding potentials without a depolarizing prepulse as illustrated in Fig. 7. Currents at test potentials of −50, −70 and −90 mV were activated from holding potentials of −60 (*a*), −40 (*b*) and −30 mV (*c*). These currents activated instantaneously; however, the slow time-dependent

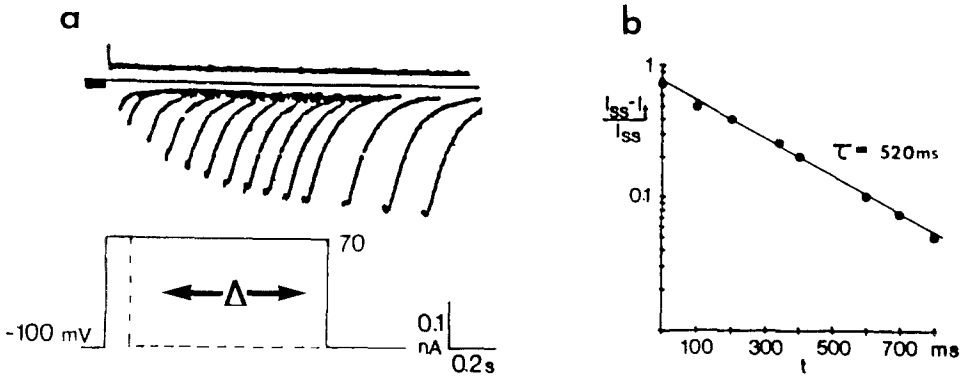

Fig. 5. (*a*) Time-dependent increase of Cs (60 mM) tail current at −100 mV as the duration of a depolarizing clamp pulse to +70 mV increases. $[Ca]_o$ = 5 mM (cell 3-15-35). (*b*) Semilogarithmic plot of the tail current envelope is a single exponential process. I_{ss} is the peak inward tail current after a 2-sec clamp pulse, and I_t is the peak inward current following a clamp pulse of duration t

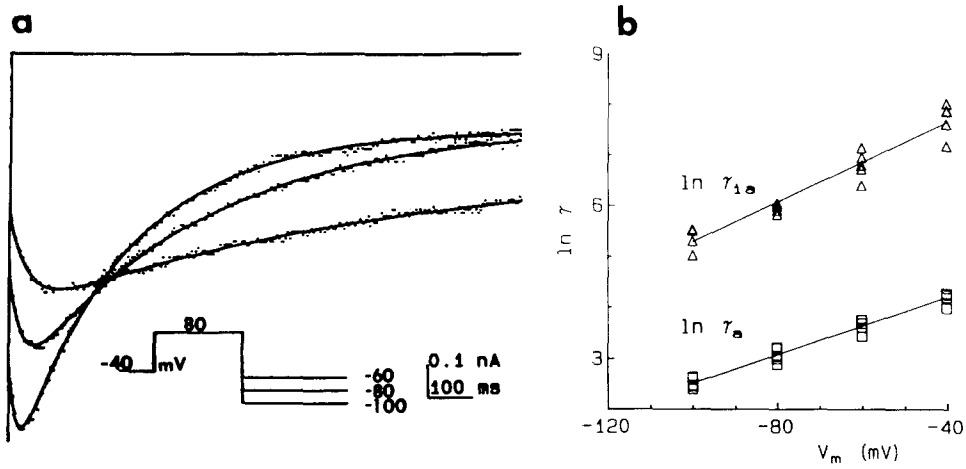

Fig. 6. Activation and inactivation of Cs (120 mM) current is the sum of two exponentials. $[Ca]_o$ = 5 mM (cell 2-16-05). (*a*) The current records (dots) are well fit by the sum of two exponentials (solid lines). The depolarizing clamp step is not shown. The equations of the curves are as follows: −60 mV, $0.28e^{-t/40} - 0.46e^{-t/832} - 0.34$; −80 mV, $0.34e^{-t/25} - 0.81e^{-t/418} - 0.22$; −100 mV, $0.3e^{-t/13.7} - 1.05e^{-t/250} - 0.24$. (*b*) Voltage dependence of ln τ_a (squares) and ln τ_{ia} (triangles) from five different cells. τ_a and τ_{ia} decreased *e*-fold per 36 and 25 mV, respectively

component of activation was not present. The holding current (arrows) and initial current jump at each test potential increased as the holding potential was made more positive, consistent with less voltage-dependent Ca block. The currents inactivate at each test potential with a similar τ_{ia}. Figure 7*d* shows the same test potentials following a 400-msec prepulse to +80 mV. The activation is characterized by an inward jump followed by slower time-dependent activation as seen in previous figures. Comparing Fig. 7*c* and *d*, the quasi-instantaneous current jump at each test potential has nearly the same amplitude; however, time-dependent activation occurs only after the prepulse to +80 mV (Fig. 7*d*). The inactivation time course is nearly identical when superimposed at the same current level. The observation that inactivation does not proceed until time-dependent activation occurs (Fig. 7*d*), despite a similar amplitude of the instantaneous inward current (Fig. 7*c* and *d*), suggests a second "gating" process occurring during the depolarizing prepulse in addition to removal of voltage-dependent Ca block.

Internal Mg Modulates Activation of I_{K1}

Since Mg has been shown to block the outward current through this channel in a time- and voltage-dependent manner (Matsuda et al., 1987; Vandenberg, 1987), the possibility that Mg affected the activation process was examined. Cs currents were recorded in the presence of a lower intracellular [Mg]

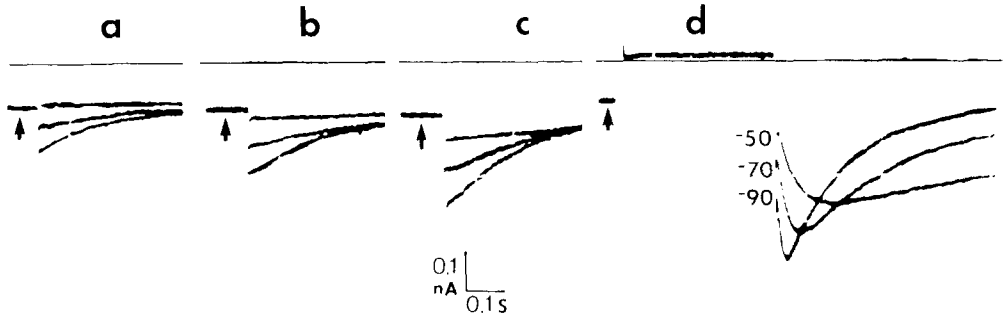

Fig. 7. Inward Cs currents (60 mM) at −50, −70 and −90 mV with (*d*) and without (*a–c*) a depolarizing prepulse. (*a*) Holding potential (H_p) is −60 mV. Initial inward current jump increases as the test potential is made more negative. Inactivation rate increases with hyperpolarization. Holding current is indicated by arrows. (*b*) H_p = −40 mV. (*c*) H_p = −30 mV. (*d*) H_p = −80 mV followed by a 400-msec pulse to +80 mV during which minimal outward current was seen. Initial current jump similar to that seen in *c*, but this is followed by activation prior to inactivation. $[Ca]_o$ = 5 mM (cell 3-28-41)

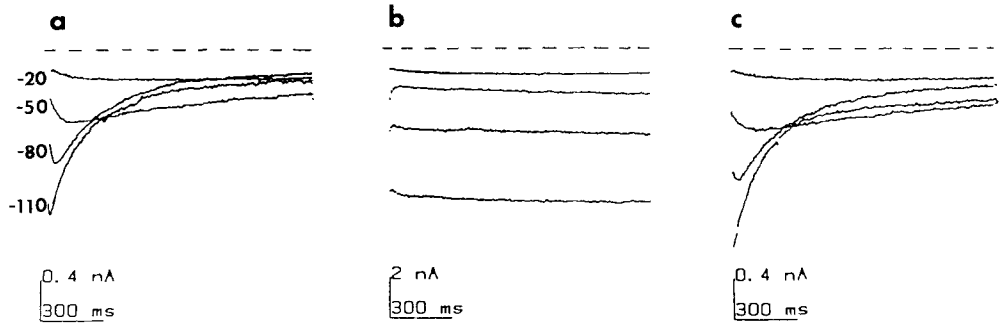

Fig. 8. Effect of external divalent cation removal on inward Cs (120 mM) currents at −20, −50, −80, and −110 mV following a 500-msec depolarizing prepulse (*not shown*) to +60 mV. Pipette Mg ≈ 50 μM buffered with the following internal solution (in mM): 50 citric acid, 45 HCl, 5 MgATP, 10 HEPES, 10 EGTA, pH 7.36 with N-methyl-D-glucamine. (*a*) External solution containing 5 mM Ca and 0.5 mM Mg. (*b*) Nonlinear currents in divalent-free external solution. Note the difference in current calibration and the loss of inactivation and marked suppression of slow activation. (*c*) Return to control solution. Cell 2-26-08

(50 μM). The currents under control conditions (Fig. 8*a*) were activated at −20, −50, −80 and −110 mV following depolarization to +60 mV (prepulse not shown). The time course was similar to currents seen in previous figures. Removal of external Ca and Mg (Fig. 8*b*) caused a four- to fivefold increase in current at each test potential and a suppression of both time-dependent activation and inactivation. Since the conductance of this current was nonlinear, it is unlikely to be a leak conductance. Return to the Ca- and Mg-containing solution (Fig. 8*c*) returned the currents to near baseline. This was seen in all cells in which the pipette [Mg] was buffered to ≈ 50 μM (n = 5). This is in marked contrast to Fig. 4 (pipette [Mg] ≈ 0.3 mM) in which removal of external Ca and Mg eliminated inactivation without significantly altering activation or the peak current amplitude. It thus appears that in the absence of external divalent cations, the presence of intracellular Mg limits both the maximum amount of inward current possible and regulates activation of the current. When $[Mg]_i$ was reduced to the micromolar range, activation and inactivation gating still occurred but only in the presence of external divalent cations.

Other Monovalent Currents through I_{K1}

Very small time-dependent currents were recorded when either Na or Li was used as the charge carrier (*data not shown*). Ammonium currents were larger than Cs but smaller than Rb currents, indicating an intermediate permeance of NH_4 ions (*data not shown*).

Comparison of Monovalent Cation Permeability through I_{K1}

The Table compares the effect of the depolarizing conditioning pulse on permeability ratios at −90 mV of various monovalent ions with respect to K. These

Table. Comparison of permeability ratios (P_x/P_K) for I_{K1}

X	P_x/P_K (−90 mV)		
	Peak inward (A)	Steady state (B)	B/A
Cs (n = 10)	0.15 ± 0.03	0.01 ± 0.003	0.07
NH_4 (n = 4)	0.30 ± 0.04	0.06 ± 0.007	0.2
Rb (n = 5)	0.51 ± 0.05	0.21 ± 0.01	0.41
Na (n = 3)	—	<0.005	
Li (n = 3)	—	<0.001	

permeability ratios are based on the Goldman-Hodgkin-Katz current equation (*see* Hille, 1984):

$$I_x = P_x z_x F \nu_x [X]_o \text{ for } V_m \ll 0 \quad (1)$$

where I_x is the current amplitude, P_x is the permeability, z_x is the valence, ν_x is the electrical mobility, and $[X]_o$ is the external concentration of ion X. F is the Faraday constant. The ratio of the currents at a given potential may reflect the permeability ratio of monovalent ion, X, to K as given by:

$$P_x/P_K = I_x/I_K \, (\nu_K/\nu_x). \quad (2)$$

ν_K/ν_x is nearly 1 for Cs and Rb (Robinson & Stokes, 1965). The Table gives the values of P_x/P_K at −90 mV calculated from experiments similar to that of Fig. 3. The ratio obtained from the peak inward current ratio is compared to that from the steady-state currents. As seen in the Table, time- and voltage-dependent block by Ca leads to varying degrees of underestimation of the monovalent cation permeability ratio in the steady state. The ratio of the steady state to the peak inward permeability (Table, third column) indicates that among the permeant ions, Cs current is most strongly blocked by external Ca followed by NH_4 and Rb. The application of Eqs. (1) and (2) to calculate permeability ratios assumes that (*i*) the permeant ion is present only on one side of the membrane (*ii*) the current is measured at a very negative potential (*iii*) the ratio of electrical mobilities within the channel are similar to those in free solution and (*iv*) independent electrodiffusion can be applied to a single-file multi-ion channel. Since the data are derived from experiments similar to that in Fig. 3, assumptions i and ii are fulfilled; however, assumptions iii and iv may not be satisfied. Despite the inherent weakness and oversimplification in applying these equations to derive permeability ratios, the steady-state values so obtained are in reasonable agreement with permeability ratios of I_{K1} based on reversal potential measurements (Hagiwara & Takahashi, 1974).

Discussion

Permeability of I_{K1} to Monovalent Cations

One of the major findings of this study is that external Ca and Mg can lead to a serious underestimation of monovalent cation permeance through the inwardly rectifying K channel due to time- and voltage-dependent block. Cs is considered impermeant through this channel with a permeability ratio of <0.03 in the starfish egg inward rectifier (Hagiwara & Takahashi, 1974) and has been shown to block this channel in oocytes, skeletal muscle and cardiac myocytes (Hagiwara, Miyazaki & Rosenthal, 1976; Gay & Stanfield, 1977; Argibay et al., 1983; Sakmann & Trube, 1984; Tourneur et al., 1987). Despite its classification as a blocking or impermeant ion, it was possible to record Cs currents through this channel. Comparison of permeability ratios derived from the steady-state currents in these experiments with those derived from reversal potential measurements in the starfish egg inward rectifier (Hagiwara & Takahashi, 1974) reveals a reasonable correlation and preservation of relative permeance: Cs (0.01 *vs.* <0.03), NH_4 (0.06 *vs.* 0.035), and Rb (0.21 *vs.* 0.35). The peak inward current ratios, however, indicate a much higher relative permeance of the cations (Table). These values may still be underestimated, since the peak inward current at −90 mV is less than the maximum possible current due to (*i*) incomplete removal of Ca block by the depolarizing prepulse and (*ii*) some degree of time-dependent Ca block that has already occurred at the peak of inward current. While we did not find evidence for Ca block of K currents ($[K]_o$ was 60 to 120 mM), Biermans et al. (1987) have demonstrated that 1.8 mM external Ca or Mg cause a marked voltage, but not time-dependent block of inward K current ($[K]_o$ = 5.4 mM). The lack of an effect in this study may be due to the higher $[K]_o$. Sakmann and Trube (1984) have reported a marked inactivation of I_{K1} at the single-channel level in cell-attached patches (150 mM pipette [K]); however, neither this study nor that of Biermans et al. (1987) could demonstrate such an intrinsic inactivation. This discrepancy may be related to differences in experimental conditions (i.e., intracellular dialysis) or due to inactivation induced by nonborosilicate glass pipettes (Cota & Armstrong, 1988).

Inactivation of I_{K1}

Time- and voltage-dependent block of I_{K1} by monovalent cations such as Cs and Na has been described in various types of tissue (Hagiwara et al., 1976; Gay & Stanfield, 1977; Ohmori, 1978; Standen &

Stanfield, 1979; Fukushima, 1982; Biermans et al., 1987). The strong voltage- and time-dependence of the monovalent block in these previous studies leads to a calculated effective electrical distance, δ, of 1.3–1.4 at the site of block. Since such a calculation assumes a single-site model, δ values greater than 1 suggest that the channel is able to accept more than one blocking ion simultaneously (Hille & Schwarz, 1978). The results reported in the present study, however, suggest that at least part of this strong voltage dependence may arise due to concurrent block by external Ca or Mg. Since Cs, Na and Li currents are most strongly blocked by external Ca, the presence of these monovalent ions in the extracellular solution may enhance the ability of external Ca to block the channel. If block were completely attributed to Ca instead of Na or Cs, the value of δ would be 0.6–0.7, which is very close to the value of δ reported for Ba block of this channel (Hagiwara et al., 1978; Standen & Stanfield, 1978). Consistent with this idea is a recent report that suggests that the Na block of I_{K1} in guinea pig ventricular myocytes is actually due to block by external or internal divalent cations (Mitra, Vereecke & Carmeliet, 1990).

Activation Rate Depends on Both Monovalent and Divalent Cations

Activation of I_{K1} has been shown to consist of an instantaneous component followed by a time-dependent monoexponential increase during hyperpolarizing clamp steps negative to E_K in oocytes (Hagiwara & Takahashi, 1974), skeletal muscle (Gay & Stanfield, 1977), and cardiac myocytes (Kurachi, 1985; Mitra et al., 1985; Harvey & Ten Eick, 1988). Is activation purely an intrinsic voltage-dependent gating process of the channel protein? The present study suggests this not to be the case. Figure 3 shows that the rate of activation is strongly dependent upon the permeant cation, being slowest for Cs and fastest for K. The voltage dependence of τ_a, however, can be fit with a single exponential which decreases e-fold per 33 to 37 mV, irrespective of extracellular [K] (Kurachi, 1985; Tourneur et al., 1987; Harvey & Ten Eick, 1988) or the monovalent cation as seen here. It thus appears that the more permeant the ion, the faster the activation kinetics. Interestingly, Tl, which is more permeant than K through I_{K1} of oocytes, also displays faster activation compared to K (Hagiwara & Takahashi, 1974; Hagiwara et al., 1977). These findings suggest that while the voltage dependence of τ_a is relatively constant, the absolute rate is influenced by the permeant cation. Decreasing internal [K] has also been shown to increase τ_a (Cohen et al., 1989).

The slow phase of activation might represent the average macroscopic rate of channel unblock as the incoming permeant ion displaces Mg or Ca from the channel into the cell. The instantaneous activation component would then represent those channels already occupied by monovalent cations at the holding potential, and therefore, immediately available to conduct following a voltage step. In this respect, the slow phase of activation in I_{K1} is reminiscent of the activation "hook" seen during repolarization in squid axon potassium channels which have been blocked by Ba. Repolarization drives external K into the channel which then serves to repel Ba from the channel into the cytoplasm (Armstrong & Taylor, 1980; Armstrong, Swenson & Taylor, 1982). These investigators found that raising external K enhanced the rate of Ba unblock at negative potentials, as would be expected in a multi-ion channel with intrachannel ionic repulsion (Hille & Schwarz, 1978). Similarly for I_{K1}, the slow phase of activation may represent the rate at which K (Rb or Cs) displaces Mg or Ca from the channel into the cell. The rate of activation would thus increase as the extracellular concentration of K (Rb or Cs) was increased. This may explain why the slow phase of activation is not reported in single-channel studies of I_{K1} which require 75 to 140 mM extracellular [K] for adequate current resolution (Kurachi, 1985; Vandenberg, 1987; Matsuda, 1988).

The role of intra- and extracellular divalent cations in gating I_{K1} has been described previously. Voltage-dependent binding of intracellular Mg in the micromolar range ($K_d \approx 1.7$ μM) is felt to be primarily responsible for inward rectification based on single-channel experiments with guinea pig ventricular myocytes (Matsuda et al., 1987; Vandenberg, 1987). Block by $[Mg]_i$ was almost instantaneous and most consistent with open-channel block. External Ca and Mg have also been shown to block at least 50% of I_{K1} channels near E_K under physiological conditions (Biermans et al., 1987). This study has shown that in the presence of 0.3 mM internal Mg, Cs currents still activate normally in the absence of external divalent cations (Fig. 4*a*). This is in marked contrast to Fig. 8 in which internal Mg is buffered to $\approx$50 μM: removal of external divalent cations results in (*i*) much larger noninactivating inward currents and (*ii*) a marked loss in slow activation. Both observations are consistent with the idea that internal Mg or external divalent cations block the channel and modulate the gating kinetics. The gating particle may be the divalent cation itself. The observation that more permeant ions display faster activation kinetics might be explained by their higher binding affinity (Latorre & Miller, 1983), and therefore, greater ability to withstand simultaneous occupancy of a chan-

nel with a divalent cation. This would also explain why weakly permeant ions, such as Cs, are blocked to a greater degree by internal and external divalent cations. Recent work by Neyton and Miller (1988) indicates that the most permeant ions through K channels (K and Rb) also have the highest binding affinities for sites within the channel.

The findings of Ishihara et al. (1989) in I_{K1} of guinea pig ventricular myocytes argue against a divalent-gating mechanism for activation as suggested above. They found the slow phase of activation gating to persist when intracellular [Mg] was buffered to 2 μM. Intrinsic gating of the inward rectifier in bovine artery endothelial cells has also been reported to be insensitive to $[Mg]_i$ buffered below 1 μM (Silver & DeCoursey, 1990). Ishihara et al. (1989), however, were unable to perform experiments with less than 2 μM $[Mg]_i$ due to instability of the preparation. Given previous estimates of a $K_d = 1.7$ μM for Mg (Vandenberg, 1987; Matsuda, 1988), there could still have been a significant effect of internal Mg on gating. The studies by Ishihara et al. (1989) and Silver and DeCoursey (1990) were also done in the presence of either extracellular Ca or Mg. As is evident in this study, even when internal Mg was buffered to the micromolar range, external Ca or Mg was able to preserve activation and inactivation gating properties (Fig. 8). This was also observed in outwardly rectifying K channels of squid neurons (Armstrong & Lopez-Barneo, 1987) and lymphocytes (Grissmer & Cahalan, 1989). Thus, the effects of lowering intracellular Mg are not fully apparent until external divalent cations are removed (Fig. 8). Armstrong and Lopez-Barneo (1987) reported that K channels in squid neurons lose their selectivity and gating in divalent free solutions. Grissmer and Cahalan (1989) observed similar phenomena: removal of all divalent cations resulted in a voltage-independent "leakage" conductance which could be prevented by the presence of as little as 1 mM Mg. They were uncertain as to whether this conductance was related to the K channel protein or was a non-specific conductance in zero-divalent solutions. An important distinction between these studies and the present one is that the increased Cs conductance is clearly voltage dependent (Fig. 8), and therefore, not likely to be a "leakage" conductance. Increased permeance of Na through I_{K1} has also been shown when intracellular [Mg] is buffered to 50 μM or less (Mitra et al., 1990). This decrease in selectivity is not associated with a loss of voltage-dependent gating.

The need for strong depolarizations to uncover activation (Fig. 7) suggest that in addition to voltage-dependent Ca unblock, another gating process occurred. If it is assumed that (*i*) external Cs can displace Mg but not Ca from the channel into the cell and (*ii*) double occupancy by divalent cations is not permitted in this channel due to the high energy of divalent-divalent repulsion, then the findings may be explained as follows: as Ca comes out of the channel with depolarization, internal Mg moves into the channel. Upon repolarization, Ca would be unable to block those channels occupied by Mg until Mg moves back into the cell. The latter process (activation) would be favored by hyperpolarization and repulsive interaction with incoming Cs ions. At negative holding potentials, fewer channels contain Mg and thus the slow phase of activation is less pronounced on hyperpolarization. The above explanation must be considered purely speculative and further work is needed to clarify these issues.

The presence of large inward Cs currents through this channel indicates that selectivity is not purely determined by a "filter" that excludes larger ions by size. It appears that steric constraints to permeance based on cation size in addition to the ability of external Ca or internal Mg to differentially impede monovalent permeance in the order: $Li > Na \gg Cs > NH_4 > Rb > K$ lead to the high selectivity towards K and play an important role in the gating properties of this channel. Futher work is needed to gain a clearer insight regarding the exact mechanisms of gating, permeance and block.

References

Argibay, J.A., Dutey, P., Ildefonse, M., Ojeda, C., Rougier, O., Tourneur, Y. 1983. Block by Cs of K currents I_{K1} and of carbachol induced current in frog atrium. *Pfluegers Arch.* **397**:295–299

Armstrong, C.M. 1981. Sodium channels and gating currents. *Physiol. Rev.* **61**:644–683

Armstrong, C.M., Lopez-Barneo, J. 1987. External calcium ions are required for potassium channel gating. *Science* **236**:712–714

Armstrong, C.M., Swenson, R.P., Taylor, R. 1982. Block of squid axon K channels by internally and externally applied barium ions. *J. Gen. Physiol.* **80**:663–682

Armstrong, C.M., Taylor, S.R. 1980. Interaction of barium ions with potassium channels in squid giant axons. *Biophys. J.* **30**:473–488

Biermans, G., Vereecke, J., Carmeliet, E. 1987. The mechanism of the inactivation of the inward-rectifying K current during hyperpolarizing steps in guinea-pig ventricular myocytes. *Pfluegers Arch.* **410**:604–613

Cohen, I.S., DiFrancesco, D., Mulrine, N.K., Pennefather, P. 1989. Internal and external K gate the inward rectifier. *Biophys. J.* **55**:197–202

Cota, G., Armstrong, C. 1988. Potassium channel "inactivation" induced by soft-glass pipettes. *Biophys. J.* **53**:107–109

Frankenhaeuser, B., Hodgkin, A.L. 1957. The action of calcium on the electrical properties of squid axons. *J. Physiol.* **137**:218–244

Fukushima, Y. 1982. Blocking kinetics of the anomalous po-

tassium rectifier of tunicate egg studied by single channel recording. *J. Physiol.* **331:**311–331

Gay, L.A., Stanfield, P.R. 1977. Cs causes a voltage dependent block of inward K currents in resting skeletal muscle fibers. *Nature* **267:**169–170

Grissmer, S., Cahalan, M.D. 1989. Divalent ion trapping inside potassium channels of human T lymphoocytes. *J. Gen. Physiol.* **93:**609–630

Hagiwara, S., Miyazaki, S., Krasne, S., Ciani, S. 1977. Anomalous permeabilities of the egg cell membrane of a starfish in K-T1 mixtures. *J. Gen. Physiol.* **70:**269–281

Hagiwara, S., Miyazaki, S., Moody, W., Patlak, J. 1978. Blocking effects of barium and hydrogen ions on the potassium current during anomalous rectification in the starfish egg. *J. Physiol.* **279:**167–185

Hagiwara, S., Miyazaki, S., Rosenthal, N.P. 1976. Potassium current and the effect of cesium ion on this current. *J. Gen. Physiol.* **67:**621–638

Hagiwara, S., Takahashi, K. 1974. The anomalous rectification and cation selectivity of the membrane of a starfish egg cell. *J. Membrane Biol.* **18:**61–80

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100

Harvey, R., Ten Eick, R. 1988. Characterization of the inward-rectifying potassium current in cat ventricular myocytes. *J. Gen. Physiol.* **91:**593–615

Hille, B. 1984. Ionic Channels of Excitable Membranes. p. 231. Sinauer, Sunderland, MA

Hille, B., Schwarz, W. 1978. Potassium channels as multi-ion single-file pores. *J. Gen. Physiol.* **72:**409–442

Ishihara, K., Mitsuiye, T., Noma, A., Takano, M. 1989. The Mg^{2+} block and gating underlying inward rectification of the K^+ current in guinea pig cardiac myocytes. *J. Physiol.* **419:**297–320

Katz, B. 1949. Les constantes électriques de la membrane du muscle. *Arch. Sci. Physiol.* **2:**285–299

Kurachi, Y. 1985. Voltage-dependent activation of the inward rectifier potassium channel in the ventricular cell membrane of guinea-pig heart. *J. Physiol.* **366:**365–385

Latorre, R., Miller, C. 1983. Conduction and selectivity in potassium channels. *J. Membrane Biol.* **71:**11–30

Martell, A.E., Smith, R.M. 1977. Critical Stability Constants. pp. 478, 651–652. Plenum, New York

Matsuda, H. 1988. Open-state substructure of inwardly rectifying potassium channels revealed by magnesium block in guinea-pig heart cells. *J. Physiol.* **397:**237–258

Matsuda, H., Saigusa, A., Irisawa, H. 1987. Ohmic conductance through the inwardly rectifying K channel and blocking by internal Mg. *Nature* **325:**156–159

Mitra, R., Morad, M. 1985. A uniform enzymatic method for dissociation of myocytes from hearts and stomachs of vertebrates. *Am. J. Physiol.* **249:**H1056–H1060

Mitra, R., Morad, M. 1987. Permeation and block of the inwardly rectifying K channel in isolated guinea pig ventricular myocytes by divalent and monovalent ions. *J. Physiol.* **382:**128P

Mitra, R., Morad, M., Tourneur, Y. 1985. Time-dependent activation of the potassium inward rectifier I_{K1} in isolated guinea-pig cardiac cells. *J. Physiol.* **358:**52P

Mitra, R., Vereecke, J., Carmeliet, E. 1990. Ca and Mg block an intrinsically high Na conductance through a cardiac K channel. *Biophys. J.* **57:**111*a*

Neyton, J., Miller, C. 1988. Potassium blocks barium permeation through a calcium-activated potassium channel. *J. Gen. Physiol.* **92:**549–567

Ohmori, H. 1978. Inactivation kinetics and steady state current noise in the anomalous rectifier of tunicate egg cell membranes. *J. Physiol.* **281:**77–99

Robinson, R.A., Stokes, R.H. 1965. Electrolyte Solutions. p. 465. Butterworths, London

Sakmann, B., Trube, G. 1984. Voltage-dependent inactivation of inward-rectifying single channel currents in the guinea-pig heart cell membrane. *J. Physiol.* **347:**659–683

Silver, M., DeCoursey, T.E. 1990. Intrinsic gating of the inward rectifier in bovine pulmonary artery endothelial cells in the presence or absence of internal Mg. *J. Gen. Physiol.* **96:**109–133

Standen, N.B., Stanfield, P.R. 1978. A potential and time-dependent blockade of inward rectification in frog skeletal muscle fibres by barium and strontium ions. *J. Physiol.* **280:**169–191

Standen, N.B., Stanfield, P.R. 1979. Potassium depletion and sodium block of potassium currents under hyperpolarization in frog sartorius muscle. *J. Physiol.* **294:**497–520

Tourneur, Y., Mitra, R., Morad, M., Rougier, O. 1987. Activation properties of the inward-rectifying potassium channel on mammalian heart cells. *J. Membrane Biol.* **97:**127–135

Vandenberg, C. 1987. Inward rectification of a potassium channel in cardiac ventricular cells depends on internal magnesium ions. *Proc. Natl. Acad. Sci. USA* **80:**2560–2564

Received 14 September 1990; revised 5 December 1990