Extracellular K⁺ Dependence of Inward Rectification Kinetics in Human Left Ventricular Cardiomyocytes

Patrick Bailly, PhD; Maud Mouchonière, PhD; Jean-Pierre Bénitah, PhD; Lionel Camilleri, MD; Guy Vassort, ScD; Paco Lorente, MD

Background—In human ventricular cells, the inwardly rectifying K⁺ current (I_{\text{Ki}}) is very similar to that of other mammalian species, but detailed knowledge about the K⁺-dependent distribution of open and blocked states during rectification and about the K⁺-dependent modulation of inactivation on hyperpolarization is currently lacking.

Methods and Results—We used the whole-cell patch-clamp technique to record I_{\text{Ki}} in myocytes isolated from subendocardial layers of left ventricular septum from patients with nonfailing hearts with aortic stenosis and cardiac hypertrophy who were undergoing open-heart surgery. Outward currents were very small at voltages positive to the reversal potential but increased at high external [K⁺]. Chord conductance measurements and kinetic analyses allowed us to estimate the proportion of channels in the open state and of those showing either slow unblock or instantaneous unblock (the so-called slow or instantaneous “activation”) on hyperpolarization; the distribution in the individual states was dependent on external [K⁺]. The proportion of channels unblocking slowly was greater than that of channels unblocking instantaneously on hyperpolarization from the plateau voltage range. Hence, because of the previously reported link between the presence of highly protonated blocking molecules and slow unblock kinetics, it is suggested that high cellular concentrations of spermine may account for the low outward current density recorded in these cells. The current decrease observed on extended hyperpolarization was significantly relieved by an increase in external [K⁺].

Conclusions—The pattern of I_{\text{Ki}} current alterations observed in the present model of human ventricular hypertrophy might favor enhanced excitability and underlie ventricular arrhythmias, possibly via increased intracellular polyamine levels.

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Key Words: potassium ■ myocytes ■ electrophysiology

The inward rectifier K⁺ current (I_{\text{Ki}}) is a major determinant of a stable resting potential in excitable cells.¹ Mediating a reduced flow of outward currents at voltages positive to reversal potential, inward-going rectification also allows development of the long plateau phase of the cardiac action potential² and sets the excitability threshold for depolarization.³ The steep inward rectification of I_{\text{Ki}} around the reversal potential (E_{\text{rev}}) is typically associated with a time- and voltage-dependent current increase after an instantaneous current jump on hyperpolarization. In the 1980s, it was shown that a voltage-dependent block by internal Mg²⁺ ions contributes to strong rectification,¹ mainly at depolarized levels far from E_{\text{rev}}.⁴ However, the fast kinetics of the Mg²⁺ block cannot account for the observed activation kinetics, because in the absence of Mg²⁺, inward rectifier K⁺ channels still rectify strongly, and the slow open-close transitions of the so-called activation gate remain little affected.⁵ Indeed, recent studies on channels exogenously expressed from cloned inward rectifier K⁺ channel genes, Kir2.1⁶ and Kir2.3,⁷ have shown that the intrinsic gating reflects the blocking and unblocking kinetics of intracellular protonated polyamines.⁸–¹³ Regulatory mechanisms are reportedly involved in these processes: on the one hand, the higher the cationic charge of the polyamine, the slower the time course of recovery from the polyamine-induced block;¹³,¹⁰ on the other hand, cloned channels, like native I_{\text{Ki}} channels, exhibit clear relief of rectification after external K⁺ concentration ([K⁺]_o) increases.¹⁰

In humans, a few recent data suggest that the characteristics of ventricular native I_{\text{Ki}} channels are similar to those of other mammalian hearts,¹¹–¹⁴ except for smaller outward current amplitudes than in other species.²,³,⁵,¹⁵ No information is currently available on the [K⁺]_o dependence of unblocking and blocking kinetics or on the conductance properties of the native human ventricular I_{\text{Ki}}. In the present article, we examine the influence of [K⁺]_o changes on the voltage dependence of nonconductive states induced by depolarizing pulses and on the “inactivation” kinetics observed during prolonged hyperpolarization in human ventricular cells from patients without left ventricular (LV) hypertrophy who were undergoing open-heart surgery. Outward currents were very small at voltages positive to the reversal potential but increased at high external [K⁺]. Chord conductance measurements and kinetic analyses allowed us to estimate the proportion of channels in the open state and of those showing either slow unblock or instantaneous unblock (the so-called slow or instantaneous “activation”) on hyperpolarization; the distribution in the individual states was dependent on external [K⁺]. The proportion of channels unblocking slowly was greater than that of channels unblocking instantaneously on hyperpolarization from the plateau voltage range. Hence, because of the previously reported link between the presence of highly protonated blocking molecules and slow unblock kinetics, it is suggested that high cellular concentrations of spermine may account for the low outward current density recorded in these cells. The current decrease observed on extended hyperpolarization was significantly relieved by an increase in external [K⁺].

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From the Département de Chirurgie Cardiovasculaire, Hôpital Gabriel Montpied, Clermont-Ferrand (P.B., L.C.), and U 390 INSERM, CHU Arnaud de Villeneuve, Montpellier (G.V., P.L.), France. Dr Bénitah is now a postdoctoral fellow in the Division of Cardiology, Johns Hopkins University School of Medicine, Baltimore, Md. Dr Mouchonière is now a postdoctoral fellow in the Institut National de Recherche Agronomique, Theix, France.

Correspondence to Dr Paco Lorente, U 390 INSERM, CHU Arnaud de Villeneuve, 34295 Montpellier, France. E-mail paco@u390.montp.inserm.fr

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dysfunction. We report that the [K+] dependence in the distribution of blocked states on depolarization in isolated human ventricular cells follows a pattern similar to that observed in Kir2.1 exogenously expressed channels. The hyperpolarization-induced “inactivation” may be accounted for both by extracellular Na+ block and by external K+ depletion, possibly facilitating a polyamine-induced channel reblock; this process seems to be significantly relieved by an increase in [K+].

Methods

Characterization of Patients

Ventricular tissue samples were obtained from 21 patients undergoing corrective cardiac surgery for acquired aortic stenosis with LV hypertrophy but without LV dysfunction. Patient age was 63.5±8.6 years (range, 48 to 78 years); 12 were men, 9 women. Clear evidence of LV hypertrophy was based on echocardiographic and ECG findings (echocardiographic septal thickness was 13.2±3.0 mm; normal values, <10 mm). All the patients had compensated hypertrophy (NYHA functional class II; cardiac index, 2.67±0.51 L min−1 m−2; ejection fraction, 0.63±0.15; LV end-diastolic pressure, 14.0±3.1 mm Hg; echocardiographic shortening fraction, 0.35±0.06) and hence were not receiving any cardiotonic drugs. All patients gave written informed consent to the study protocol, which had previously been approved by the institutional committee on human investigation and complies with principles outlined in the Declaration of Helsinki.

Cell Isolation

Endocardial LV septal sampling was performed through the aortic orifice during circulatory arrest. Specimens were taken from the coronary leaflets of the aortic valve. They consisted of scalpel shavings from the LV septal wall (3 mm thick, 0.1 to 0.6 cm3), in every case superficial (3-mm depth). Immediately after the specimens were taken, they were placed in cardioplegia solution bubbled with 100% O2 at 19°C.

Experimental procedures began within 15 minutes of removal. Ventricular myocytes were isolated by an enzymatic dissection method as described previously. Briefly, chunks were incubated at 35°C for 30 minutes in a Ca2+-free Tyrode’s solution supplemented with 300 IU/mL collagenase V, 4 IU/mL protease XXIV, and 1 mg/mL BSA (Sigma). Then, the supernatant was removed and replaced by a fresh enzyme medium having the same composition but without protease. When the yield appeared to be maximal, minced tissue was strained through a 200-μm nylon mesh to remove debris and undigested tissue. Cells were then suspended in Ca2+-free Tyrode’s solution and stored for 1 hour at room temperature (20°C to 22°C) before the experiment was begun. Only quiescent rod-shaped cells showing clear striations without significant granulation were used.

Recording Techniques

Current recordings were obtained by the standard whole-cell recording technique with an Axopatch 1D amplifier (Axon Instruments) with a 100-MΩ feedback resistance headstage, the 125-kHz Labmaster board, and pClamp program V 5.5.1 (Axon Instruments). Microelectrodes pulled from soft glass capillary tubing (1.5 to 1.6 mm OD) had tip resistances ranging from 1 to 1.5 MΩ. A silver–silver chloride pellet encased in a 3 mol/L KCl agar bridge was placed in the bath and used as the ground reference electrode. Cell capacitance and series resistance were measured by ±10-mV voltage steps applied from a −70-mV holding potential and calculated as previously described. Series resistance was kept <5 MΩ (2.26±1.04 MΩ; n=42) and was compensated by 60% to 80%; the time constant of the capacitive current decay was 0.19±0.04 ms after compensation. The electrode potential was adjusted to zero after immersion of the pipette tip; this zeroing caused a positive voltage bias that was not corrected. Currents were low-pass filtered at 2 kHz, digitized at a sampling interval of 200 μs, and stored for off-line analysis.

Solutions

The transport solution contained (in mmol/L) NaCl 147, KCl 20, CaCl2 2, MgCl2 16, glucose 6, and HEPES 5 (pH adjusted to 6.8 with KOH). For cell isolation and cell storage, the Ca2+-free Tyrode’s solution had the following composition (in mmol/L): NaCl 120, KCl 4, MgCl2 1, HEPES 10, and glucose 6; pH was adjusted to 7.4 with NaOH. For study of Kir1 kinetics and gating properties, the standard Tyrode’s solution contained (in mmol/L) NaCl 130, KCl 4, CaCl2 2, MgCl2 1.1, mannitol 0.4, HEPES 25, and glucose 11; pH was adjusted to 7.4 with NaOH. In experiments designed to study the [K+] dependence of inward rectification kinetics, different [K+], were set by equimolar substitution of KCl for NaCl in the Tyrode’s solution. Patch pipettes were filled with an internal solution containing (in mmol/L): KCl 120, MgCl2 1, Mg-ATP 3, Tris-GTP 0.4, EGTA 10, HEPES 25, and glucose 10 (pH adjusted to 7.2 with KOH). The addition of EGTA buffer to the internal solution aimed at minimizing calcium-activated outward currents and the absence of Na+ ions in the internal solution were also expected to inhibit calcium influx through Na+-Ca2+ exchange. Ca2+ current was inhibited by 2 mM of L Co2+. L was inhibited by 3 mM of L-aminopyrindine, and Na+ current was minimized by the voltage-clamp protocol.

Kinetic Analysis

The results are expressed as mean±SEM. As recently reported by Ishihara, current kinetics relative to channel states were analyzed at the end of depolarizing pulses, as follows. The proportion of channels residing in the open state (P0) was estimated from the chord conductance, G, calculated as the current level: driving force ratio G=I/Vm(Vm−E0). Then, G was normalized to its maximum value, Gmax, obtained at Vm=−130 mV, which was ~50 mV negative to E0; P0=G/Gmax. Single Boltzmann fits were used to describe the voltage dependence of P0.

It was clear from tail currents at −130 mV after depolarizing steps that channels were in at least 2 nonconductive states at the end of the preceding potential: one that opened instantaneously on hyperpolarization (the proportion of channels in this state was called PC,slow) and another that activated in a time-dependent manner (the proportion of channels in this state was called PC,fast). The analysis of inward currents recorded by hyperpolarizing the membrane from various levels to −130 mV allowed us to obtain PC,slow and PC,fast. The time-dependent increase of inward currents was best fitted by a single exponential function of the form A exp[−(t−k)/τ]+C, and the current level at the onset of voltage change, I(0), was obtained by extrapolating back the fitted curve to the beginning of the test pulse. The amplitude of the time-dependent component relative to the maximum level C of the inward current at −130 mV, [C−I(0)]/C, was assumed to give an estimate of PC,slow, at the membrane potential that preceded hyperpolarization. On the basis of previously reported works, we hypothesized that PC,slow represented the proportion of channels blocked by the most positively charged polynomials just before hyperpolarization.

An estimation of the probability of the channel being in the nonconductive state that opens instantaneously on hyperpolarization was provided by P0[(I(0)/C)]−E0, i.e., the fraction of instantaneous outward currents not attributable to channels open at the end of previous conditioning potentials. We hypothesized that PC,slow represented the proportion of channels blocked by the least positively charged polynomials and by Mg2+ ions just before hyperpolarization.

In contrast, the current decline during prolonged hyperpolarizations was best fitted by a double exponential function of the form A1 exp[−(t−k)/τ1]+A2 exp[−(t−k)/τ2]+B, where τ1 and A1 are the time constant and initial amplitude of the fast component, τ2 and A2 the time constant and initial amplitude of the slow component, and B the steady-state component.

Subsequently, “activation” and “inactivation” are written in quotes because they describe increase and decrease in current during voltage steps but do not refer to their classic meaning.

Results

The cell membrane was held at −50 mV (Vh), ~40 mV positive to the predicted K+ equilibrium potential (Eh).
Currents were elicited by 200-ms voltage-clamp steps applied from $V_h$ to $-170$ to $-20$ mV at 0.16 Hz. After a 5-ms return to $V_h$, a 50-ms voltage-clamp pulse was delivered to $-140$ mV. Inward rectification was evidenced by larger current amplitudes at voltage steps negative to $-80$ mV (Figure 1A). The “activation” phase of the inward current speeded up as voltage steps were made more negative. A significant decay of inward current (“inactivation”) occurred at voltages negative to $-100$ mV, and marked declines at stronger hyperpolarizations caused a negative slope region in the steady-state relation (Figure 1B). $E_{rev}$ was between $-80$ and $-70$ mV, and for less negative potentials, very small outward currents gave rise to a flat zone without any negative slope conductance; on the average, the maximum outward current density was $0.57 \pm 0.15 \text{ pA/pF}$ at $-50$ mV.

Increasing $[K^+]_o$ induced a depolarizing shift in $E_{rev}$, an increase in the peak current and slope conductance, and a slight increase of outward currents in 20 mmol/L $[K^+]_o$ (Figure 2A). Linear fitting to mean $E_{rev}$ plotted against log $[K^+]_o$ yielded a slope of $51.5 \pm 3.0$ mV, close to the $58.5$ mV expected from the Nernst equation (Figure 2B), thus suggesting that the main charge carriers for the background current in human ventricular cells are $K^+$ ions. The square-root dependence of conductance of inward rectifiers on $[K^+]_o$ was also a property of the human ventricular macroscopic $I_{K1}$ (Figure 2C): slope conductances measured from the linear portion of the I-V relationship at potentials negative to $E_{rev}$ were plotted versus $[K^+]_o$, and fitted by a linear regression function. The mean slope of regression lines obtained from individual cells was $0.54 \pm 0.19$ (n=11).

“Activation” (Unblock) Kinetics
As in native and cloned channels, a depolarizing shift in $E_{rev}$, an increase in the peak current and slope conductance, and a slight increase of outward currents in 20 mmol/L $[K^+]_o$ (Figure 2A). Linear fitting to mean $E_{rev}$ plotted against log $[K^+]_o$ yielded a slope of $51.5 \pm 3.0$ mV, close to the $58.5$ mV expected from the Nernst equation (Figure 2B), thus suggesting that the main charge carriers for the background current in human ventricular cells are $K^+$ ions. The square-root dependence of conductance of inward rectifiers on $[K^+]_o$ was also a property of the human ventricular macroscopic $I_{K1}$ (Figure 2C): slope conductances measured from the linear portion of the I-V relationship at potentials negative to $E_{rev}$ were plotted versus $[K^+]_o$, and fitted by a linear regression function. The mean slope of regression lines obtained from individual cells was $0.54 \pm 0.19$ (n=11).

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increased when prepulse levels were successively depolarized from −100 to −50 mV. Conversely, the proportion of channels blocked by the least protonated molecules, $P_{C\text{Inst}}$, corresponds to the value $1 - P_{C\text{Slow}} - P_O$, where $P_O$ is the probability of the channel being opened in the preceding membrane potential. From the I-V relations obtained at the end of 20-ms prepulses, the voltage dependence of $P_O$ was estimated at different $[K^+]_o$ successively applied (Figure 4B). Increasing $[K^+]_o$ augmented $P_O$ (ie, decreased channel block) at any given membrane potential and caused an obvious $P_O$ shift to more positive potentials. To describe the $[K^+]_o$ dependence of inward rectification, the membrane potential at which channels were half-blocked ($V_{1/2}$) was obtained from single Boltzmann fits to experimental $P_O$ values, and its dependence on $E_K$ was examined. In 4, 8, and 20 mmol/L $[K^+]_o$, $V_{1/2}$ and $E_K$ mean values were (in mV) −92.3, −90.4; −74.2, −72.8; and −48.5, −49.5, respectively. Hence, inward rectification shifted proportionally to the change in $E_K$, and a linear approximation to these data gave a slope factor of 1.07. $P_{C\text{Slow}}$ and $P_{C\text{Inst}}$ at the end of prepulses were estimated from the kinetic analysis of currents in the following hyperpolarization (Figure 4C and 4D). Steep increases in $P_{C\text{Slow}}$ were observed at potentials around $E_K$ ($−90$ and $−73$ mV at 4 and 8 mmol/L $[K^+]_o$, respectively), but $P_{C\text{Slow}}$ decreased only to a moderate extent at more depolarized potentials. $P_{C\text{Inst}}$ increased in a voltage-dependent manner at both $[K^+]_o$, but did not cross over the $P_{C\text{Slow}}$-voltage relationship within the voltage range studied, in contrast to previously reported findings.6

“Inactivation” Kinetics on Hyperpolarization

The time-dependent decline of $I_{K_1}$ on hyperpolarization has been referred to as “inactivation.”25,28,29 As previously reported,28 the best fit to current traces was also provided in our cells by 2 exponentials demonstrating a clear dependence on voltage. On average, at $−120$, $−130$, $−150$, and $−170$ mV, the time constants of the fast and slow components, $\tau_f$ and $\tau_s$, were (in ms) 30, 243; 25, 135; 16, 53; and 11, 33, respectively.

The $[K^+]_o$ dependence of “inactivation” kinetics was investigated in cells successively bathed in 4, 8, and 20 mmol/L $[K^+]_o$, and using voltage steps to $−170$ mV (Figure 5A). As $[K^+]_o$ was raised, both peak ($I_{\text{peak}}$) and steady-state ($I_s$) currents were increased and the time course of “activation” was accelerated. However, “inactivation” was slowed down, resulting in a decrease of the relative amount of current “inactivated” at the end of the pulse. If we define the difference between the peak and steady-state current as $I_{\text{inact}}$, then the $I_{\text{inact}}/I_{\text{peak}}$ ratio was 0.80, 0.63, and 0.23 at 4, 8, and 20 mmol/L $[K^+]_o$, respectively. This behavior was observed in 5 of 5 cells studied.

It was also of interest to examine the classic steady-state inactivation relationship (Figure 5B). Normalized currents plotted versus prepulse potentials were well fitted with the function $I/I_{\text{max}} = 1/(1 + \exp[(V_{1/2} - V_m)/k]) + B$, where $B$ is the steady-state baseline level. In 4 and 8 mmol/L $[K^+]_o$, $V_{1/2}$ ($V_m$ for $I/I_{\text{max}} = 0.5$) and $k$ were (in mV) $−154.8 ± 2.1$, $8.1 ± 1.1$ mV and $−161.9 ± 1.3$, $11.0 ± 1.3$, respectively; in 20 mmol/L $[K^+]_o$, $k$ was $22.2 ± 2.8$ mV. For potentials more negative than $−140$ mV, the availability was higher with increasing $[K^+]_o$.
the calculated steady-state baseline level was 0.19±0.02, 0.31±0.03, and 0.60±0.05 in 4, 8, and 20 mmol/L [K⁺]o, respectively.

The time-dependent decrease of Iκ1 during hyperpolarizing pulses was attributed in part to extracellular K⁺ depletion in isolated feline ventricular myocytes. The notion that depletion may also occur in human ventricular myocytes was tested with a 2-pulse tail protocol in 4 mmol/L [K⁺]o (Figure 5C). Erev deduced from tail-current measurements was compared with that obtained from the steady-state I-V relationship. At the end of the conditioning pulse, there was a −8.2-mV shift of Erev relative to the one determined from the steady-state I-V relationship. On average, the negative shift of Erev related to extracellular K⁺ depletion amounted to −7.0±1.3 mV (n=7). Assuming that intracellular K⁺ activity does not change, this shift in Erev corresponded to a decrease of 1.1 mmol/L in [K⁺]o (Figure 2B). From the expected change in conductance resulting from this [K⁺]o decrease (Figure 3C) and the consequent change in driving force, we may expect a current decline of ≈24% at −170 mV caused by depletion of extracellular K⁺ in the unstirred solution layer close to the cellular surface. Because the difference between peak and steady state at −170 mV shown in Figure 1B suggests a mean decrease of 73% in Iκ1 conductance, depletion might account for ≈33% of the reduction in Iκ1 conductance at −170 mV.

Discussion

The major findings of the present study are threefold. (1) The nonconductive states in human ventricular Iκ1 channels follow a [K⁺]o dependence analogous to that observed in expressed Kir2.1 channels. (2) The distribution pattern of blocked states suggests a more prominent binding to high-affinity than to low-affinity blocking molecules within the plateau voltage range. (3) There is clear evidence that increasing [K⁺]o may relieve the “inactivation” induced by strong hyperpolarizations.

[K⁺]o Dependence of Iκ1 Conductance in Human Ventricular Myocytes

The square-root dependence of conductance on [K⁺]o, a property of native and cloned inward rectifier channels from mammalian tissues, Exogenous expressions of cloned channels have demonstrated that inward currents are substantially inhibited by polyamines over the full range of voltages negative to EK, raising the question of possible alterations of inward conductance in native channels as well. Therefore, the conductance-[K⁺]o relationship may depend on several polyamine-related factors. Indeed, an approximate square-root dependence can be found in macroscopic native currents (eg, in the present study), possibly because conductance measurements are performed at [K⁺]o, much lower than the expected internal K⁺ concentration. In this case, it is recognized that Iκ1 channels behave as unsaturated open-channel pores at potentials negative to Ek and the square-root dependence of conductance on [K⁺]o just reflects this behavior.

[K⁺]o Dependence of Unblocking Kinetics

We previously showed that action potential duration (APD) is prolonged in human LV septal cells from nonfailing hypertrophied hearts, and we attributed this pattern partially to a dramatic downregulation of Iκ1. However, outward Iκ1 currents also contribute to the final phase of repolarization. Accordingly, the rate of late repolarization, which was found to be slower in hypertrophied cells than in control cells, prompted us to search for alterations in Iκ1 current. We found only very weak outward currents at potentials positive to Erev compared with those reported in other mammalian species. This was in contrast to the observations of Koumi et al but consistent with other studies on human ventricular cells and may suggest the presence of high cytoplasmic polyamine levels and particular distributions of Iκ1 channel states on depolarization.

The assumed distribution of channels in the individual state was apparently dependent on [K⁺]o. Since the P O fitted curve reflected the voltage dependence of inward rectification, the [K⁺]o-dependent shift of inward rectification was estimated by the V 1/2=EK relationship, which demonstrated high sensitivity on [K⁺]o, because the slope between V 1/2 and EK was 1.07, a value close to that observed for spermine in inside-out patches from Xenopus oocytes expressing Kir2.1. The voltage and [K⁺]o dependence of P O and P S were reminiscent of that established for the proportion of channels blocked by Mg²⁺ (P Mg) and spermine (P Spm) in murine fibroblast cells, with a positive shift of ≈20 mV by increasing [K⁺]o, from 4 to 8 mmol/L. However, in contrast to the latter reported findings, no relationship crossover was found; the fraction of channels blocked by highly protonated molecules remained greater than the fraction of channels blocked by weakly protonated molecules within the plateau voltage range. This pattern must be interpreted by considering the reported ability of Mg²⁺ and putrescine (Put²⁺) to increase outward currents as follows. Given that Put²⁺ and Mg²⁺ blocking rate constants are larger than that of spermine, Put²⁺/Mg²⁺-blocked states can reach higher proportions than spermine-blocked states (P Spm) at short depolarizing voltage steps. Thereafter, the highest affinity of spermine with the channel induces time-dependent redistribution of Put²⁺/Mg²⁺-blocked to spermine-blocked states, and this occurs through an intermediate open state. As a result, the larger the number of Mg²⁺/Put²⁺-blocked channels are, the more channels reside in the open state before passing into the spermine-blocked state, thus increasing outward current amplitude. However, because blocking rates depend on the concentrations of blocking molecules, an increase in spermine concentration may entail negative and upper shifts of P Spm at the expense of P Mg/Put and thus reduce outward currents because of the competitive binding of blocking molecules to the channel. Therefore, the higher values of P S within the plateau voltage range compared with P S, int might be indicative of relatively high levels of spermine concentration, underlying decreased availability of outward current and hence enhanced excitability.

[K⁺]o Dependence of Current “Inactivation”

Increasing [K⁺]o apparently relieves Iκ1 “inactivation” during hyperpolarizing pulses (Figure 5A). The steady-state “inactivation” shown in Figure 5B also favors the notion that high [K⁺]o, significantly alleviates “inactivation,” because after...
“inactivation” has emerged from the use of various Na\(^+\) substitutes subsequently considered inhibitory\(^{28}\) or even as open-channel blockers.\(^{32}\) Indeed, it is unlikely that [Na\(^+\)], acts as an open-channel blocker, but it can redistribute channels to a closed state, thus contributing to the decline of current during prolonged hyperpolarization.\(^{25}\) This may be reconciled with the multiple-ion-block hypothesis that suggests interactions between permeant and blocking ions at multiple binding sites.\(^{1}\) Accordingly, Na\(^+\)/K\(^+\) competition in the channel pore may be an important mechanism by which increasing [K\(^+\)], can relieve “inactivation.”

Extracellular K\(^+\) depletion may also account for some decrease in \(I_{k1}\) conductance during hyperpolarization\(^{6,7}\) and is presumably amplified by the structure of the T-tubular system. The K\(^+\) selectivity filter, cradled at the external mouth of the pore,\(^{10}\) and the deep, high-affinity binding sites for polyamines probably underlie the key structures governing the selective K\(^+\) conduction in \(I_{k1}\) channels through electrostatic interactions.\(^{20}\) Thus, the extracellular K\(^+\) depletion induced by hyperpolarization might reduce K\(^+\) occupancy at the selectivity filter and thus decrease interactions with polyamine binding sites; this should allow partial re-block of the channel by polyamines and might contribute to the “inactivation” process. Because relative changes of extracellular K\(^+\) in restricted spaces are expected to be less important in high than in low [K\(^+\)], this “interactive” mechanism might also contribute to the relief of “inactivation” induced by high [K\(^+\)], together with the Na\(^+\)/K\(^+\) competition mentioned above.

**Physiological Implications**

Extended depolarization decreases outward current by strengthening high-affinity spermine block.\(^{6,10}\) A depolarizing shift of \(V_h\) also reduces it by increasing \(P_{\text{perm}}\) and diminishing the availability of channels to be blocked by Mg\(^{2+}\) on depolarization.\(^{6}\) Therefore, APD prolongation usually observed in the present model\(^{10}\) or slight resting potential depolarizations can reduce the proportion of Mg\(^{2+}\)/Put\(^{+}\)-blocked channels during the plateau, then decreasing the outward current flow during repolarization and further lengthening APD. Conversely, recent reports\(^{15,17}\) have demonstrated that manipulation of polyamine levels can bring about dramatic changes in the repolarization phase and significantly increase excitability. Hence, \(I_{k1}\) alterations during evolving cardiac hypertrophy in patients with aortic stenosis may favor enhanced excitability and arrhythmias, insofar as elevated polyamine levels have been found in hypertrophy models.\(^{17}\)

In other aspects, the question remains whether the depolarizing shift of the voltage dependence of blocked states induced by [K\(^+\)], increases might contribute to the APD lengthening observed at the initial stage of acute myocardial ischemia.\(^{24}\) Indeed, the possibility cannot be ruled out that the cellular K\(^+\) loss subsequent to acidification at the early stage of acute ischemia may entail a rightward shift of blocked-state relationships. A subsequent increased prevalence of high-affinity polyamine-blocked states in the plateau voltage range might be expected from the observed patterns (Figure

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**Figure 5.** [K\(^+\)],-induced relief of “inactivation.” A through C, Cells are successively exposed to 4 (○), 8 (□), and 20 (●) mmol/L [K\(^+\)]. A, Currents elicited in 1 representative cell (\(C_m=80\) pF) by a −170-mV pulse followed by a pulse to −140 mV (\(V_h=−50\) mV). Bottom 2 dashed lines show amplitude of current “inactivated” (\(I_{\text{inact}}\)) during test pulse at 20 mmol/L [K\(^+\)], calculated as difference between peak (\(I_{\text{peak}}\)) and steady-state (\(I_{\text{ss}}\)) currents. For clarity, currents “inactivated” at 4 and 8 mmol/L [K\(^+\)], are not represented. B, Steady-state “inactivation” of inward current studied with 2-pulse protocol shown in inset. Currents measured 2 ms after onset of test pulse (TP) were normalized to maximum value obtained with conditioning pulses (CP) that totally removed “inactivation” and were plotted against CPs. C, Steady-state current-voltage relation established with protocol shown in Figure 1 (C), together with steady-state (■) and instantaneous (□) tail currents obtained with a 2-pulse tail protocol. Inset, Currents elicited in a cell during later-protocol protocol applied to determine \(E_{\text{Ko}}\); from a −50-mV \(V_h\), prepulse was to −170 mV for 80 ms, and subsequent test pulses were applied to −120 to −10 mV in 5-mV increments. Tail currents were extrapolated by fitting to beginning of test pulses. \(C_m=112\) pF. Steady-state currents reversed at 8.2 mV less negative than tail currents.

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strong hyperpolarizing pulses, the current availability is ~3-fold greater in 20 than in 4 mmol/L [K\(^+\)]. The time-dependent decline of inward \(I_{k1}\) on hyperpolarization in physiological solution was classically ascribed to a voltage-dependent block of the channel by external Na\(^+\), reportedly found in native and cloned inward rectifiers.\(^{9,25,29}\) This view is supported by the disappearance of the negative slope in the steady-state current-voltage relationships when Na\(^+\) substitutes are used.\(^{25,28,29}\) However, this phenomenon may develop in the absence of external Na\(^+\) in guinea-pig ventricular cells\(^{13}\) and in Kir2.3 channels expressed in *Xenopus* oocytes.\(^{14}\) In contrast, the notion of [Na\(^+\)], block–induced
4), with a resulting decrease in outward current availability and AP prolongation.

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