

Characterization of the Inward-rectifying Potassium Current in Cat Ventricular Myocytes

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ABSTRACT Whole-cell membrane currents were measured in isolated cat ventricular myocytes using a suction-electrode voltage-clamp technique. An inward-rectifying current was identified that exhibited a time-dependent activation. The peak current appeared to have a linear voltage dependence at membrane potentials negative to the reversal potential. Inward current was sensitive to K channel blockers. In addition, varying the extracellular K⁺ concentration caused changes in the reversal potential and slope conductance expected for a K⁺ current. The voltage dependence of the chord conductance exhibited a sigmoidal relationship, increasing at more negative membrane potentials. Increasing the extracellular K⁺ concentration increased the maximal level of conductance and caused a shift in the relationship that was directly proportional to the change in reversal potential. Activation of the current followed a monoexponential time course, and the time constant of activation exhibited a monoexponential dependence on membrane potential. Increasing the extracellular K⁺ concentration caused a shift of this relationship that was directly proportional to the change in reversal potential. Inactivation of inward current became evident at more negative potentials, resulting in a negative slope region of the steady state current-voltage relationship between -140 and -180 mV. Steady state inactivation exhibited a sigmoidal voltage dependence, and recovery from inactivation followed a monoexponential time course. Removing extracellular Na⁺ caused a decrease in the slope of the steady state current-voltage relationship at potentials negative to -140 mV, as well as a decrease of the conductance of inward current. It was concluded that this current was I_{K1} , the inward-rectifying K⁺ current found in multicellular cardiac preparations. The K⁺ and voltage sensitivity of I_{K1} activation resembled that found for the inward-rectifying K⁺ currents in frog skeletal muscle and various egg cell preparations. Inactivation of I_{K1} in isolated ventricular myocytes was viewed as being the result of two

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processes: the first involves a voltage-dependent change in conductance; the second involves depletion of K^+ from extracellular spaces. The voltage-dependent component of inactivation was associated with the presence of extracellular Na^+ .

INTRODUCTION

Since Katz (1949) first discovered the property of anomalous rectification in skeletal muscle, it has been described in several different preparations. This anomalous or inward rectification has been studied extensively in starfish (Hagiwara and Takahashi, 1974; Hagiwara et al., 1976; Hagiwara and Yoshii, 1979) and tunicate (Miyazaki et al., 1974; Ohmori, 1978, 1980; Fukushima, 1982) egg cells as well as skeletal muscle preparations (Adrian et al., 1970; Almers, 1972*a, b*; Standen and Stanfield, 1979; Hestrin, 1981; Leech and Stanfield, 1981). Inward rectification is associated with the presence of a background K ion conductance that contributes to the maintenance of the resting membrane potential. In addition, many of the characteristics of the K^+ current produced upon hyperpolarization are similar in these preparations. The current is composed of a time-independent or instantaneous component as well as a time-dependent component. The time-dependent portion can be separated into two processes: (*a*) a K^+ - and voltage-dependent activation, and (*b*) a voltage-dependent inactivation associated at least partially with the presence of extracellular Na^+ .

An inward-rectifying K^+ current (I_{K1}) has also been described in cardiac ventricular tissue, but it is considered to be an instantaneous current that is not associated with any time dependence (Beeler and Reuter, 1977; McDonald and Trautwein, 1978; Cleeman and Morad, 1979). This may be due to the technical difficulties associated with detecting I_{K1} . Multicellular preparations require the use of voltage-clamp techniques with slower response times, and the large preparations result in larger, more slowly decaying capacitative transients. These factors impair the resolution of current during the first few milliseconds of a voltage-clamp step. For these reasons, most studies have assumed I_{K1} to be the K^+ -dependent background current remaining after subtraction of the slower time-dependent components from the total membrane current (McDonald and Trautwein, 1978; Cleeman and Morad, 1979). However, recent reports (Sakmann and Trube, 1984*a, b*; Kurachi, 1985; Mitra et al., 1986; Harvey and Ten Eick, 1987; Pennefather et al., 1987) suggest that it is no longer tenable to regard the cardiac inward-rectifying K^+ current as a time-independent background current. With the development of cell-isolation techniques and newer single-cell voltage-clamping methods, it is now possible to study this current directly. The experiments described here indicate that the inward-rectifying K^+ current in isolated cat ventricular myocytes, measured using a single-suction-electrode voltage-clamp technique, exhibits many similarities to the inward-rectifying K^+ current elicited in skeletal muscle and egg cells. Preliminary results have been published in abstract form (Harvey and Ten Eick, 1987).

METHODS

Preparation of Myocytes

The methods used to isolate adult cat ventricular myocytes are a modification of those originally described by Silver et al. (1983); they have been described in detail elsewhere (Follmer et al., 1987). Briefly, hearts were rapidly excised and coronary vessels were retrogradely perfused after cannulation of the aorta. After 2–3 min of perfusion with a nominally Ca²⁺-free Krebs-Henseliet buffer solution (KHB), the heart was perfused with KHB containing 0.15% class II collagenase (CooperBiomedical, Inc., Malvern, PA). After 30–40 min, perfusion was stopped. Ventricular and atrial tissue were separated, minced, and incubated in a shaker bath for 5–10 min in collagenase-containing solution. The remaining tissue pieces were removed by filtering, and cells were washed free of collagenase and stored in KHB containing 1% albumin and 1 mM Ca²⁺. Some cells were further maintained in primary culture for 1–2 d. No differences between K⁺ currents in freshly isolated and cultured cells were observed.

Voltage-Clamp and Recording Technique

Whole-cell membrane currents were measured using the patch-clamp technique in the whole-cell configuration described by Hamill et al. (1981). Suction pipettes were made using borosilicate glass capillary tubing (0.8–1.1 mm i.d.) and a two-stage vertical pipette puller. When pipettes were filled with internal solution, their tip resistances ranged between 0.5 and 1.0 M Ω . When they were placed in external solution, their liquid junction potentials ranged between –7 and –10 mV. Data were not compensated for this effect.

Current recordings were obtained using a whole-cell voltage-clamp circuit (Narahashi et al., 1987). Inverted voltage-clamp pulses were applied to the bath while the cell interior was maintained at ground potential. Membrane currents passing through the pipette were recorded via a current-to-voltage converter (3523L operational amplifier [Burr-Brown Corp., Tucson, AZ] with a 10-M Ω feedback resistor). Current recordings were filtered with a 1.8- or 2.5-kHz low-pass filter and recorded digitally using a PDP 11/73 computer (Digital Equipment Corp., Marlboro, MA).

To ensure adequate voltage-clamp control, the series resistance (R_s) between the current-to-voltage converter and the cell membrane was carefully compensated for by adding a portion of the current output to the command pulse. R_s was estimated from calculations of cell capacitance and the time constant of the capacitive current decay (see Follmer et al., 1987). The series resistance after compensation was 0.55 ± 0.23 M Ω (SD, $n = 25$), accounting for 70% of the total R_s before ringing occurred. The cells used in these experiments had a peak current of <6 nA at –140 mV, under control conditions. This would result in a voltage drop of ≤ 3 mV across the series resistance.

The time constants for the time-dependent current changes were estimated using a nonlinear sum of the least-squares fitting routine. Simultaneous fits to both the rising and falling phases were made when appropriate, and the time course of the current was extrapolated to time zero. Because of the voltage sensitivity of the time constant of activation (see Results), errors due to the voltage drop across the series resistance would be <5%. This estimate is based on the larger currents evoked in 20 mM external K⁺. Under control conditions, this error would be expected to be smaller. All results are reported as means \pm SD.

Solutions

The control external solution was a standard HEPES-buffered modified Tyrode's solution with the following composition (millimolar): 140 NaCl, 5.4 KCl, 2.5 CaCl₂, 0.5 MgCl₂, 5.5 HEPES, and 5.0 glucose. In experiments where extracellular concentrations of Na⁺ or K⁺ were changed, [Na⁺]_o was varied between 0 and 127.5 mM and [K⁺]_o was varied between 2 and 20 mM. The other constituents were the same as in the control solution, and the osmolarity was balanced with tetramethylammonium (TMA) chloride. The pH of the external solutions was adjusted to 7.4 using either NaOH or TMA hydroxide. The pipette or internal solution contained (millimolar): 130 K-glutamate, 10 KCl, 10 NaCl, 1.0 ATP-K₂, 1.0 MgCl₂, and HEPES 5.0. The pH of the pipette solution was adjusted to 7.2 using KOH. All experiments were performed at 32 ± 0.5°C.

RESULTS

Identification of I_{K1}

The voltage-clamp protocol used was expected to cause a change in only the background current during hyperpolarization. The cell membrane was held at -40 mV, which is 45 mV positive to the predicted K⁺ equilibrium potential under these conditions ([K⁺]_o = 5.4 mM). Fig. 1 A shows current produced by a test pulse to -100 mV. Rather than being purely voltage dependent and having only an instantaneous component, the current also exhibited time dependence. After decay of the capacitive transient, there was a time-dependent increase in the inward current leading to a steady state. The level of steady state inward current (~1.45 nA) at 15 mV negative to the reversal potential for K⁺ was much larger than the outward holding current (~0.58 nA). With one-third the driving force, there was 2.5-fold more current in the inward direction, consistent with the idea that this was an inward-rectifying K⁺ current, possibly I_{K1} .

Two families of currents are shown in the insets in Fig. 1 B. Inset *b* shows currents elicited during test pulses between -40 and -110 mV. This presumably represents the inward-rectifying K⁺ current. As the membrane potential was stepped to more negative levels, the time-dependent and steady state current became more inward. The inward-rectifying nature of the current is illustrated in the current-voltage (*I-V*) relationship, shown in Fig. 1 B. The peak current, measured as the maximal inward current, shows inward-rectifying properties negative to the holding potential. The current reversed at -88 ± 3.4 mV (*n* = 32). At test potentials positive to the holding potential, the peak current became inward again. This probably represents activation of a Ca²⁺ current. The size of this current was variable and it tended to wane with time, presumably owing to the loss of some internal constituent necessary for Ca channel function, caused by internal dialysis with the pipette solution (Irisawa and Kokubun, 1983).

The current measured at the end of hyperpolarizing test pulses shows rectifying properties similar to the peak current. Because there was no detectable difference between the current measured at 75 ms and the current measured at the end of much longer test pulses (1 s), the former appeared to represent the steady state current at these potentials and is referred to as such. The lack of a

region of negative slope conductance between -60 and -20 mV in the steady state I - V relationship is consistent with the background current found in multicellular cat ventricular preparations (Trautwein and McDonald, 1978). Also consistent with the behavior expected of a background current was the fact that varying the prepulse potential did not significantly alter the steady state current level (see Fig. 9 A).

The effect of various channel blockers was examined to determine the ionic nature of the current. The K channel blockers Cs^+ and Ba^{2+} inhibited the cur-

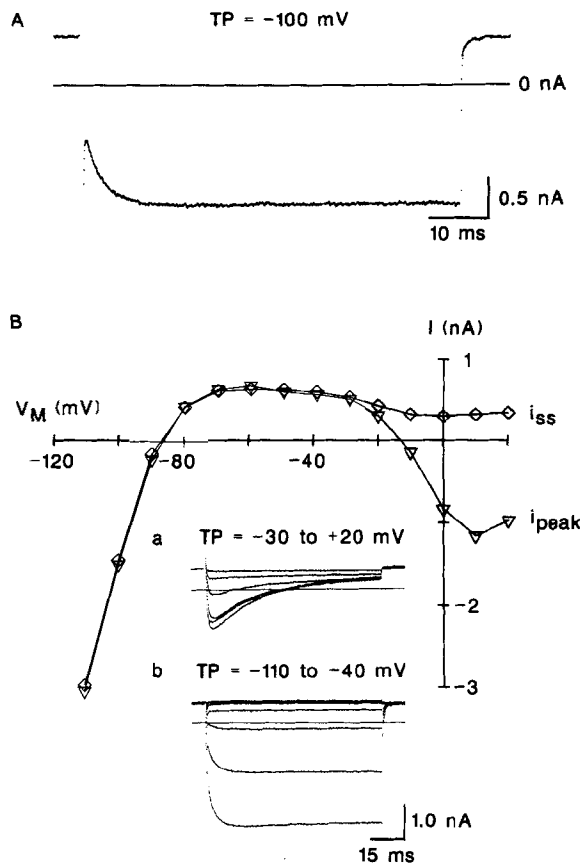


FIGURE 1. (A) Whole-cell membrane current elicited by a voltage-clamp test pulse (TP) to -100 mV from -40 mV holding potential. (B) I - V relationship of peak (i_{peak}) and steady state (i_{ss}) current. (Insets) Current elicited at membrane potentials from -30 to $+20$ mV (a) and -110 to -40 mV (b) in 10 -mV steps. $[\text{K}^+]_o = 5.4$ mM.

rent elicited upon hyperpolarization. Fig. 2 A shows the effect of 5 mM Cs^+ added to the extracellular solution. The inset illustrates Cs^+ block of the current at -110 mV. At the onset of the test pulse, there appeared to be an instantaneous inward jump of current, somewhat obscured by the capacitive transient, followed by a very rapid (<2 ms) decrease of the current to a steady state level that was net outward. The steady state I - V relationship shows that inward current could not be elicited even by test pulses as negative as -110 mV. The outward current at more positive potentials appeared to be less significantly

affected, as expected when Cs^+ is applied only extracellularly (Matsuda and Noma, 1984). The application of the Ca channel blocker Cd^{2+} had little effect on the current between -40 and -110 mV (Fig. 2 *B*).

The reversal potential was determined in various external K^+ concentrations to verify the ionic composition of the inward-rectifying current. Fig. 3 *A* shows current traces resulting from voltage-clamp pulses to -100 mV from a holding potential of -40 mV in 2, 5, 10, and 20 mM $[\text{K}^+]_o$. In 2 mM $[\text{K}^+]_o$, -100 mV is positive to the reversal potential (E_K), and the current is outward. At the higher $[\text{K}^+]_o$, -100 mV is negative to E_K , resulting in inward current. Increasing $[\text{K}^+]_o$ increased the inward current almost uniformly. When $[\text{K}^+]_o$ was

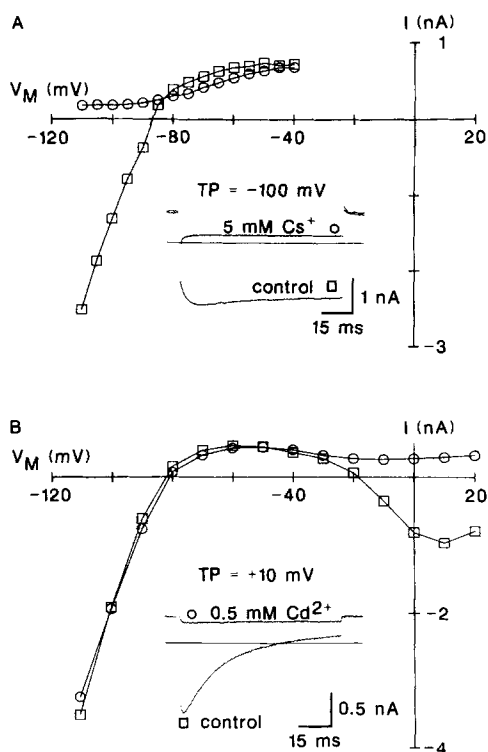


FIGURE 2. (A) Effect of Cs^+ on steady state membrane current: I - V relationship of steady state current before and after the addition of 5 mM Cs^+ to the external solution. The inset shows the effect of Cs^+ on current during a test pulse from -40 to -100 mV. (B) Effect of Cd^{2+} on peak membrane current: I - V relationship of peak current before and after addition of 0.5 mM Cd^{2+} to the external solution. The inset shows the effect of Cd^{2+} on current during a test pulse from -40 to $+10$ mV. $[\text{K}^+]_o = 5.4$ mM.

increased, the initial instantaneous jump increased, as did the peak and steady state current level, and a shortening of the time course of activation was evident. Fig. 3 *B* shows the peak I - V relationship measured in a cell exposed to several values of $[\text{K}^+]_o$. The reversal potentials measured correspond well with the values predicted for a pure K^+ current. Fig. 4 *A* shows a plot of $\log [\text{K}^+]_o$ vs. the membrane potential at which the current reversed. The points were fitted by a straight line using the Nernst equation for a pure K^+ current, which under these conditions has a slope of -60.3 mV.

It appears that this inward-rectifying K^+ current is I_{K1} , even though it exhibits an unexpected time dependence. This current also resembles the time-depen-

dent inward-rectifying K⁺ currents found in skeletal muscle and egg cell preparations. Therefore, further experiments were performed to see whether I_{K1} in cat ventricular myocytes exhibits additional characteristics resembling those reported in the other preparations.

K⁺ Sensitivity of the I_{K1} Slope Conductance

In egg cells (Hagiwara and Takahashi, 1974; Ohmori, 1978; Fukushima, 1982), a square-root dependence has been observed between $[K^+]_o$ and conductance. Therefore, the K⁺ sensitivity of the I_{K1} slope conductance was examined. The

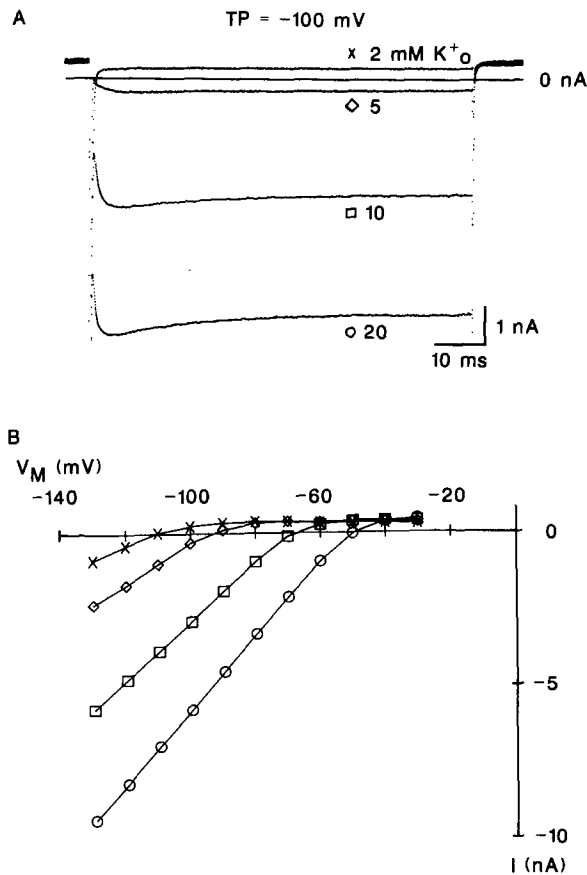


FIGURE 3. Effect of changing $[K^+]_o$ on membrane current. (A) Current elicited by a voltage-clamp pulse (TP) to -100 mV from a holding potential of -40 mV at 2, 5, 10, and 20 mM $[K^+]_o$. (B) I - V relationships of peak current during exposure to the same $[K^+]_o$ levels as in A.

peak and steady state I - V relationships were linear at membrane potentials negative to E_K . Therefore, the slope conductance of each component was determined by calculating the slope of the linear portion of the I - V relationship at potentials negative to the current reversal potential. Plotting the I_{K1} conductance as a function of $[K^+]_o$ revealed a linear logarithmic relationship with a slope of 0.56 (Fig. 4 B), which indicates that the conductance of I_{K1} is approximately proportional to the square root of $[K^+]_o$.

K⁺ and Voltage Dependence of the I_{K1} Chord Conductance

In addition to showing a K⁺ sensitivity, the chord conductance of noncardiac preparations also exhibits a voltage dependence that is related to the reversal potential of the current. For this reason, the voltage, as well as the K⁺ sensitivity of I_{K1}, was examined. Fig. 5 A shows the chord conductance (g_{K1}) as a function of membrane potential at various external K⁺ concentrations. The potential

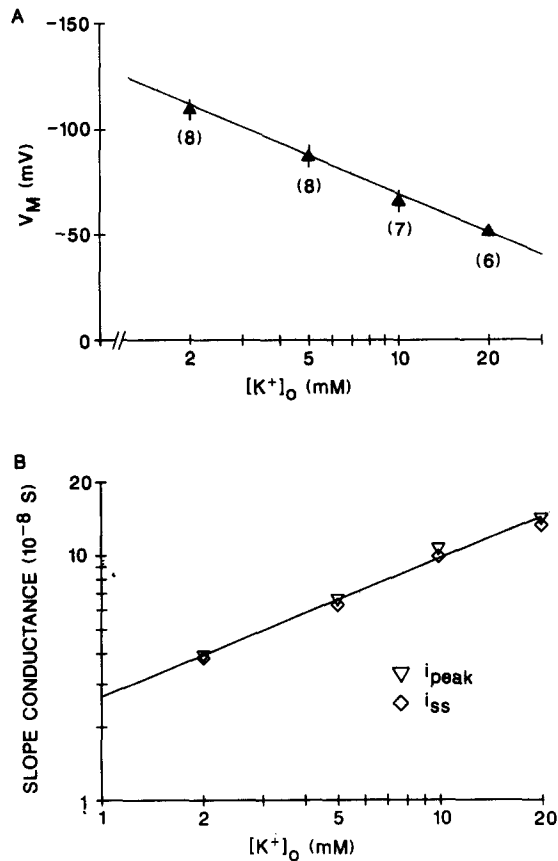


FIGURE 4. Dependence of reversal potential and slope conductance on [K⁺]_o. (A) Semilogarithmic plot of membrane current reversal potential determined from *I-V* relationships at 2, 5, 10, and 20 mM [K⁺]_o. The number of cells for which the reversal potential was determined at each concentration is given in parenthesis. The vertical lines represent standard deviations. The line was calculated from the Nernst equation and predicts the reversal potential for a pure K⁺ current, at 32°C. The slope is -60.3 mV. (B) Logarithmic plot of slope conductance as a function of [K⁺]_o. Conductance was calculated from the slope of the linear portions of the peak and steady state *I-V* relationships at voltages negative to the reversal potential. Data are from one representative cell. The line was fitted by computer to the data points using a sum of least-squares fitting program and has a slope of 0.56.

dependence of conductance exhibited a sigmoidal relationship, increasing as the membrane potential became more negative, with g_{K1} being approximately half-maximal at E_K. Increasing [K⁺]_o increased conductance at negative membrane potentials, and shifted the voltage-conductance relationship to more positive potentials. When conductance is plotted as a function of the test potential (V_M) minus the measured E_K (Fig. 5 B), the conductances at each selected [K⁺]_o reach

a minimum and maximum at approximately the same voltage levels. This indicates that I_{K1} conductance in cat ventricular myocytes depends on $[K^+]_o$ and the membrane potential where the membrane potential is described by $V_M - E_K$. This is similar to that found for inward-rectifying K⁺ currents in starfish egg cells (Hagiwara et al., 1976; Hagiwara and Yoshii, 1979) and frog skeletal muscle (Hestrin, 1981; Leech and Stanfield, 1981).

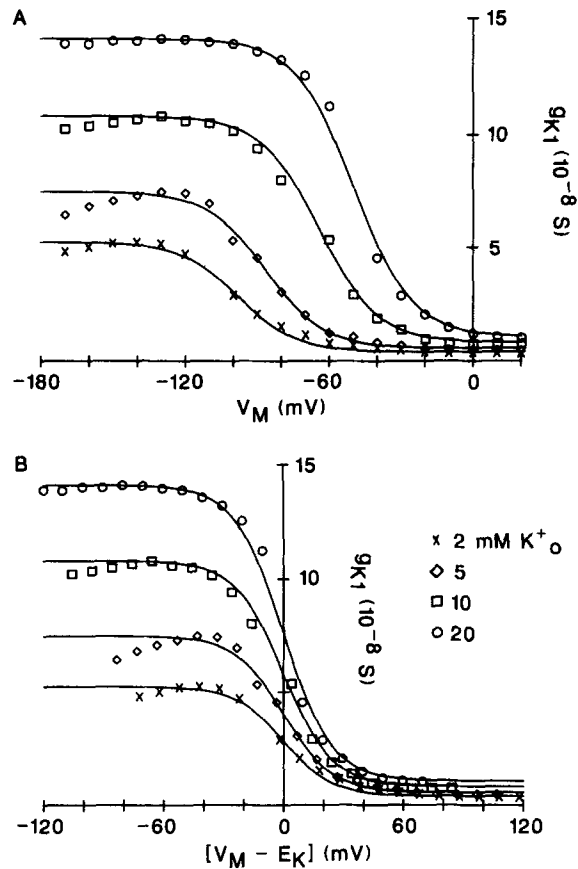


FIGURE 5. K⁺ and voltage dependence of chord conductance (g_{K1}). The chord conductance was calculated using the equation $g_{K1} = I / (V_M - E_K)$, where I = peak current except at potentials positive to -40 mV, where steady state current was used. (A) g_{K1} plotted as a function of voltage-clamp test potential at 2, 5, 10, and 20 mM $[K^+]_o$. (B) Data in A replotted as a function of membrane test potential relative to the measured current reversal potential. Data points were fitted by a least-squares sigmoidal curve-fitting program.

K⁺ and Voltage Dependence of I_{K1} Activation

In both starfish egg cells (Hagiwara et al., 1976) and frog skeletal muscle (Hestrin, 1981; Leech and Stanfield, 1981), activation of the inward K⁺ current follows an exponential time course, and the rate of this activation exhibits an exponential voltage dependence, becoming faster at more negative potentials. In addition, a K⁺ sensitivity of activation is evident as a shift of the voltage dependence related to the change in E_K . Therefore, it was of interest to examine the K⁺ and voltage dependence of I_{K1} activation.

In isolated myocytes, the time course of the increasing inward current could be approximated by a single exponential, τ_A . When the time course of the

inward current was extrapolated back to the beginning of the test pulse, no instantaneous inward current could be detected when $[K^+]_o$ was 5.4 mM. This is probably due to the fact that g_{K1} was reduced to a very low level by a holding potential well positive to E_K (see Fig. 5A). It is evident from Fig. 1B that the rate of activation of the current was accelerated at more negative potentials. When $[K^+]_o$ was 5.4 mM, the time constant of activation was 2.82 ± 0.29 ms ($n = 7$) at -90 mV and decreased exponentially to 1.03 ± 0.04 ms ($n = 3$) at -140 mV (see Fig. 6A). Fig. 6B shows the results from one experiment, where

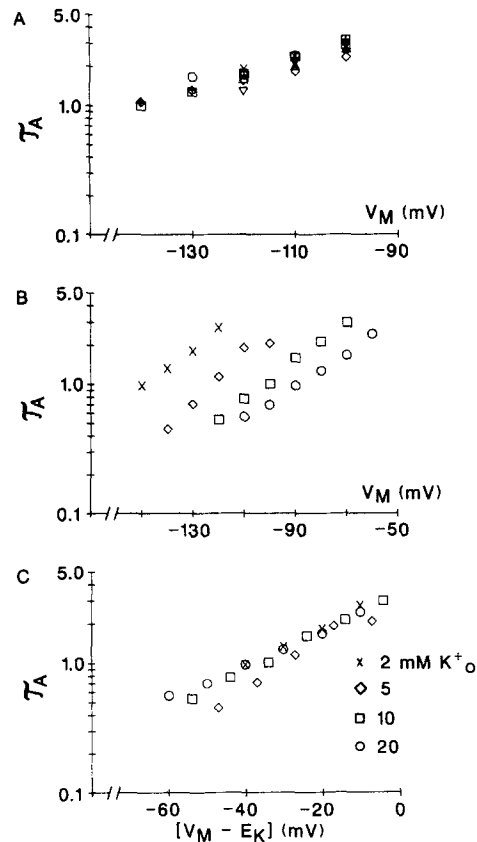


FIGURE 6. K^+ and voltage dependence of the inward current activation time course. (A) Semilogarithmic plot of the potential dependence of the time constant of activation (τ_A) for cells exposed to 5.4 mM $[K^+]_o$. Each symbol represents data obtained from a different cell. (B) Semilogarithmic plot of the potential dependence of τ_A from a cell exposed to 2, 5, 10, and 20 mM $[K^+]_o$. (C) Data in B replotted as a function of the membrane test potential relative to the measured current reversal potential.

the voltage dependence of τ_A was examined at four different $[K^+]_o$ concentrations. Increasing $[K^+]_o$ shortened the time constant of activation at a given test potential. This was associated with a more or less parallel depolarizing shift of the relationship. As with the K^+ and voltage sensitivity of the chord conductance, when the time constants at various $[K^+]_o$ values are replotted as a function of $(V_M - E_K)$ (Fig. 6C), to account for the shift in the reversal potential caused by changing $[K^+]_o$, the data points appear to fall along the same line in the semilogarithmic plot. This indicates that the sensitivity of the activation rate

to $[K^+]_o$ and membrane potential is similar to that of the conductance of the inward rectifier.

Inactivation of I_{K1}

In tunicate egg cells and frog skeletal muscle, current activated upon hyperpolarization also undergoes a time-dependent decline or inactivation, which at more negative potentials results in a negative slope region of the steady state I - V relationship. For this reason, I_{K1} was examined at more negative potentials. Although the inward-rectifying K^+ current activates with an increasingly rapid

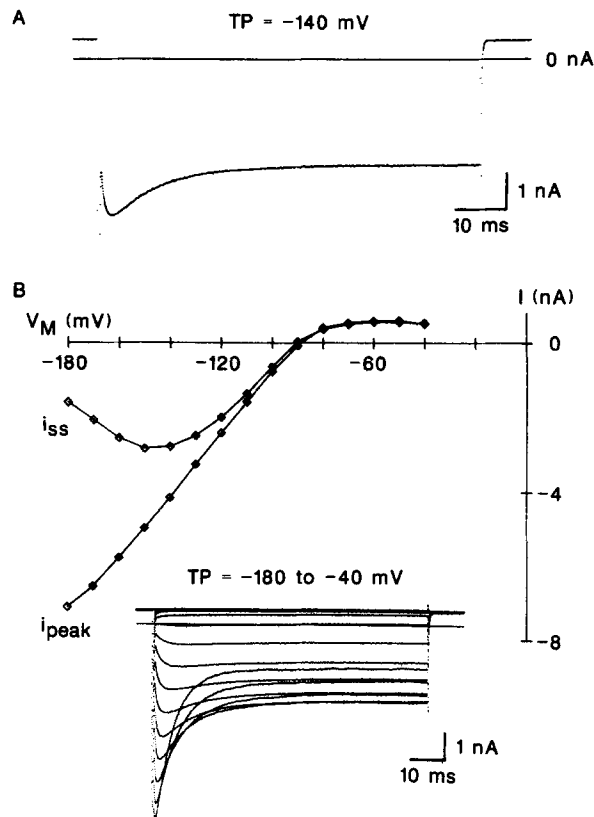


FIGURE 7. (A) Membrane current elicited by a voltage-clamp test pulse to -140 mV from a holding potential of -40 mV, showing a time-dependent decline of inward current following the activation phase. (B) I - V relationships of the peak and steady state currents. The inset shows currents elicited at membrane test potentials (TP) between -180 and -40 mV, from a holding potential of -40 mV, in 10 -mV steps. $[K^+]_o = 5.4$ mM.

time course at membrane potentials more negative than -120 mV, it also undergoes a subsequent time-dependent decline. The current evoked at -140 mV (see Fig. 7A) illustrates this phenomenon. As the membrane was hyperpolarized to potentials as negative as -180 mV, the peak current became more inward. In contrast, the steady state current continued to increase only to potentials around -140 mV, then the level of the steady state current became progressively less inward (Fig. 7B). Note that the peak I - V relationship is relatively linear negative to E_K , whereas the steady state I - V relationship is approximately

linear between E_K and -130 mV, but exhibits a negative slope region negative to -140 mV.

K⁺ Dependence of Inactivation

One explanation for the time-dependent decrease of I_{K1} may be that extracellular K^+ in the unstirred fluid layer near the surface of the cell is depleted by large inward K^+ currents, causing a decrease in conductance owing to its K^+ dependence. This process has been found to be partially responsible for the decline of K^+ current in skeletal muscle (Adrian et al., 1970; Almers, 1972*a, b*; Standen and Stanfield, 1979). Even though isolated myocytes were used to minimize this problem, the possibility that depletion was occurring cannot be ruled out. The observation that g_{K1} did not reach a stable plateau at the maximum conductance level (see Fig. 5) encourages caution in this regard. Therefore, the effect of changing $[K^+]_o$ on inactivation was examined.

Increasing $[K^+]_o$ was shown previously to cause a proportional increase in the peak current (Fig. 3*B*). Changing $[K^+]_o$ has a similar effect on the size of the steady state current (Fig. 8). It should be noted that despite the change in E_K , the potential at which the negative slope region begins is not affected by changing $[K^+]_o$. This is in contrast to the effect of changing $[K^+]_o$ on the chord conductance and the activation time course, both of which show a shift related to the change in E_K . The process or processes responsible for the inactivation that results in the negative slope region of the I - V curve at voltages negative to -140 mV appear to be unaffected by changes in $[K^+]_o$. This would place the idea that depletion alone causes the current decline in some doubt.

The possibility that depletion was occurring at membrane potentials more negative than -140 mV was tested directly using a two-pulse protocol to determine E_K after a 75-ms conditioning pulse to -170 mV. Fig. 8*B* shows the results from one such experiment performed when $[K^+]_o$ was 5.4 mM. The tail current was measured immediately after the decay of the capacitive transient. The I - V relationship of this current indicated that E_K at the end of the hyperpolarizing pulse to -170 mV had changed; the reversal potential after 70 ms at -170 mV was -97 ± 4.6 mV. This represents a shift of -7.1 ± 1.4 mV relative to the reversal potential determined from the steady state I - V relationship ($n = 6$). Assuming that intracellular K^+ activity does not change appreciably, this shift corresponds to a change in $[K^+]_o$ from 5.6 to 3.75 mM (see Fig. 4*A*). Taking into account the expected change in I_{K1} conductance (see Fig. 4*B*) and the change in driving force, depletion would be expected to cause a current decline of $\sim 27\%$ at -170 mV. However, the difference between peak and steady state current at -170 mV suggests that the conductance decreases $66 \pm 1.7\%$ ($n = 6$). Therefore, depletion accounts for $<40\%$ of the reduction in I_{K1} conductance at -170 mV.

Voltage Dependence of Inactivation

Almers (1972*a, b*) characterized the decline of the inward K current in frog skeletal muscle and separated it into two processes: (*a*) a time-dependent change in

conductance caused by K⁺ depletion and (b) a time- and voltage-dependent change in permeability. For this reason, the voltage dependence of *I_{K1}* inactivation was examined to see if it might explain the decline of inward current not associated with the depletion of extracellular K⁺ that occurs during strong hyperpolarizations in cat ventricular myocytes.

Fig. 9 illustrates the steady state voltage dependence of inactivation characterized using the two-pulse protocol depicted in the inset of Fig. 9 B. Prepulses to selected levels between -220 and -80 mV were applied for 200 ms to allow

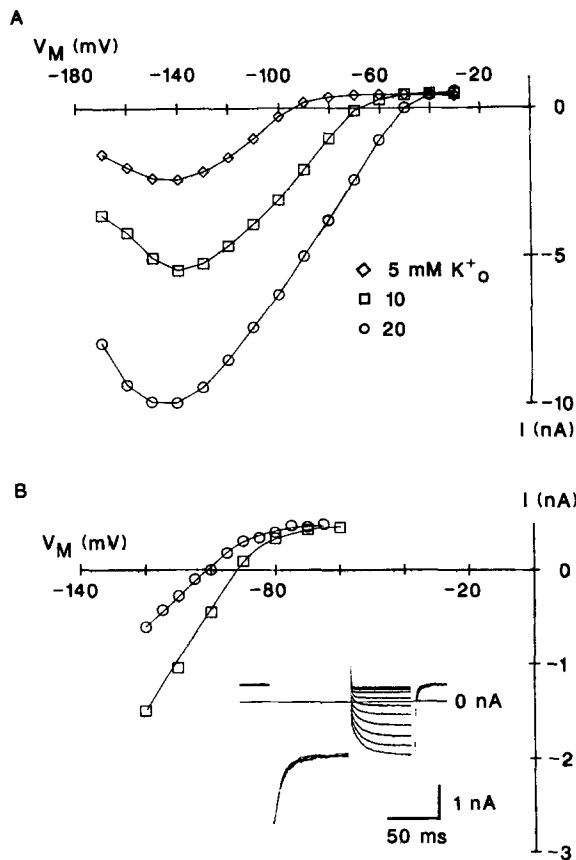
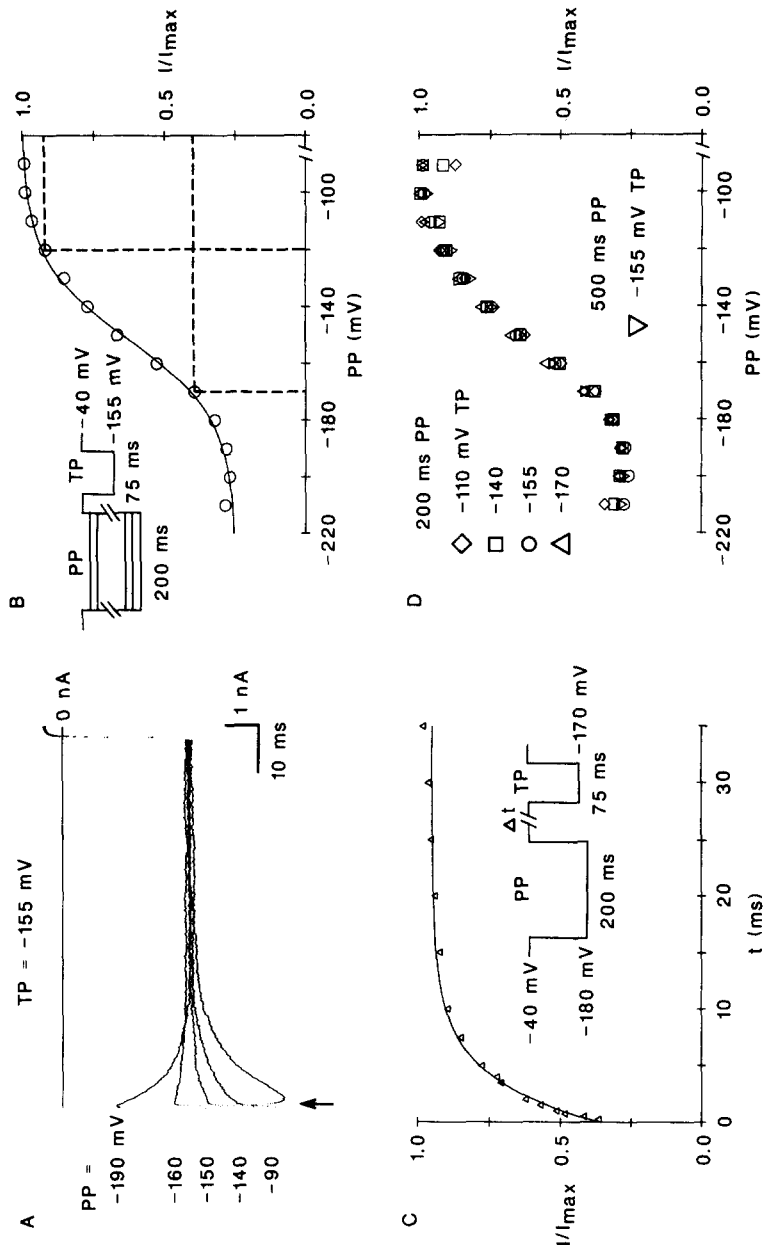


FIGURE 8. (A) Steady state *I-V* relationships obtained from a cell exposed to 5, 10, and 20 mM [K⁺]_o. (B) *I-V* relationships of the steady state (squares) and instantaneous tail (circles) currents. The inset shows currents elicited during the two-pulse protocol used to determine *E_K* following a hyperpolarizing conditioning prepulse. The prepulse was to -170 mV for 75 ms and the subsequent test pulses were to potentials between -120 and -65 mV in 5-mV increments, also for 75 ms. The tail current was measured immediately after the decay of the capacitive transient. [K⁺]_o = 5.4 mM.

the current to reach a steady state, and the current elicited during a subsequent test pulse to -155 mV was measured. The amount of pseudo-instantaneous current (measured 1.3 ms after the beginning of the test pulse) depended on the degree of hyperpolarization during the prepulse; the steady state current level was essentially unaffected by the prepulse potential. When the normalized currents were plotted vs. the prepulse potential (Fig. 9 B), the result showed a sigmoidal relationship. The current was never completely inactivated by a hyper-



polarizing prepulse. It reached a steady state level of $28 \pm 4.0\%$ ($n = 10$) of the level of control when prepulse potentials were negative to -200 mV; the test current was maximal when prepulse potentials were positive to -100 mV. The relationship shows an e-fold change every 13.4 ± 1.7 mV ($n = 10$). The membrane potential at which steady state inactivation reached half-maximum ($V_{1/2}$) was -157 ± 6.9 mV ($n = 10$). Because $V_{1/2}$ was very close to the potential used during the test pulse, further experiments were performed to determine the importance of the test pulse potential as well as the duration of the conditioning prepulse. Fig. 9D shows the results from a typical experiment where steady state inactivation relationships were determined using test potentials of -110 , -140 , -155 , and -170 mV following conditioning prepulses of either 200 or 500 ms duration. Similar data were obtained from three other experiments. From this it was concluded that the shape and position of the steady state inactivation curve were not dependent on either the test pulse potential or the prepulse duration. These results quantitatively and qualitatively resemble the voltage-dependent changes in permeability of frog skeletal muscle described by Almers (1972b), which indicates that a voltage-dependent process dominates I_{K1} inactivation in isolated cat ventricular myocytes.

Additional evidence that depletion of extracellular K⁺ plays a relatively minor role in the inactivation of I_{K1} responsible for the negative slope region of the steady state I - V relationship is found in Fig. 9B. Because the steady state current level at -120 and -170 mV was approximately the same (see Fig. 7B), the degree of depletion would be expected to be similar. However, the amount of steady state inactivation was not (Fig. 9B). This is consistent with the idea that the inactivation process responsible for the negative slope region cannot be due solely to depletion of K⁺ from extracellular spaces (Adrian et al., 1970).

FIGURE 9. (*opposite*) Steady state inactivation and recovery from voltage-dependent inactivation of inward current under control conditions ($[K^+]_o = 5.4$ mM). (A) Current elicited during the test pulse portion of a protocol used to evaluate the extent of steady state inactivation produced by 200-ms prepulses (PP) to the indicated voltages. Current was measured 1.3 ms (arrow) after the beginning of the test pulse. (B) Plot of normalized current elicited by a test pulse (TP) to -155 mV following a 200-ms conditioning prepulse to various potentials. A nonlinear least-squares fitting program was used to generate the line through the data points. From this, the steady state baseline level, the potential at which current was half-maximal, and the slope factor of the data were determined. These values were used for comparison of data obtained from different cells and different protocols. The dashed lines indicate the level of voltage-dependent inactivation at -170 and -120 mV. (C) Time course of recovery from steady state inactivation induced by 200-ms prepulses to -180 mV evaluated from current elicited in response to a -170 -mV test pulse applied at selected times after the conditioning prepulses. The points were fitted to a monoexponential function by a sum of least-squares fitting program. (D) The voltage dependence of steady state inactivation is independent of the potential (TP) at which the degree of inactivation was evaluated. The test pulse potentials and prepulse durations used for voltage-clamp protocols resembling that shown in the inset of B are indicated by the various symbols. Data are from one representative cell.

Recovery from Voltage-dependent Inactivation

The time course of recovery from inactivation at -40 mV (Fig. 9 C) was determined using a two-pulse protocol, in which an inactivating prepulse to -180 mV was followed by a test pulse to -170 mV. The prepulses and test pulses were separated by recovery periods at a holding potential ranging from 0.25 to 35 ms. The current measured 1.3 ms after the onset of the test pulse was normalized to the fully recovered current and plotted as a function of time. The time course of recovery could be fitted by a monoexponential function with a time constant of 4.0 ± 0.7 ms ($n = 4$). In skeletal muscle, recovery of the inward-rectifying K^+ current from voltage-dependent inactivation also follows a monoexponential time course (Almers, 1972b).

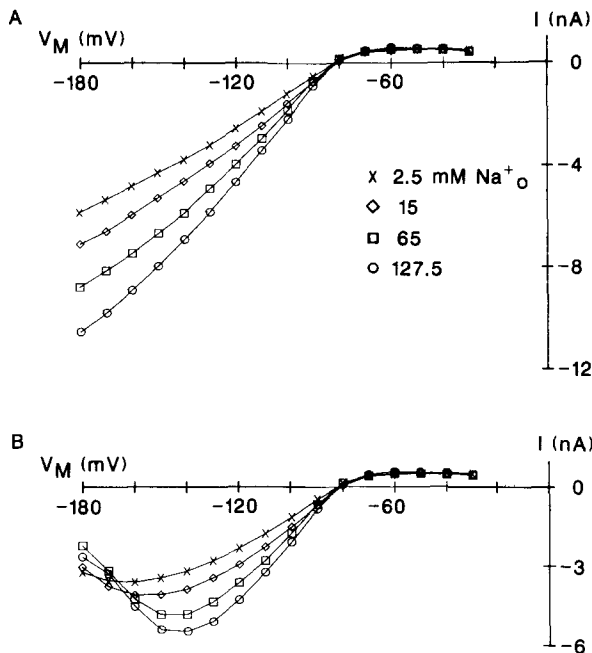


FIGURE 10. Effect of varying $[Na^+]_o$ on the peak (A) and steady state (B) *I-V* relationships of a representative cell. 2.5, 15, 65, and 127.5 mM $[Na^+]_o$ were used; $[Na^+]_o$ was replaced with TMA.

Na^+ Dependence of Inactivation

In tunicate egg cells (Ohmori, 1978, 1980; Fukushima, 1982) and frog skeletal muscle (Standen and Stanfield, 1979), the inactivation of the inward-rectifying K^+ current is altered by removing Na^+ from the extracellular solution. To determine the importance of extracellular Na^+ to the inactivation of I_{K1} , $[Na^+]_o$ was replaced with TMA. Fig. 10 shows the effect of $[Na^+]_o$ on the peak and steady state *I-V* relationships in a cell exposed to 5 mM K^+ . Removing Na^+ had two effects: it decreased the peak amplitude of I_{K1} , and it linearized the steady state *I-V* relationship. The size of the peak current shows a direct relationship to $[Na^+]_o$ (Fig. 10 A). Removing $[Na^+]_o$ reduced the magnitude of the current with-

out affecting the reversal potential. The effect of $[\text{Na}^+]_o$ on the steady state current (Fig. 10 B) was similar at membrane potentials between E_K and -140 mV. Beyond -140 mV, the dependence of the steady state current magnitude on Na^+ seemed to disappear. Fig. 11 shows the $[\text{Na}^+]_o$ dependence of current elicited by test pulses to two different potentials. At -120 mV, a potential at which inactivation is not significant, changing $[\text{Na}^+]_o$ had a uniform effect on peak and steady state current. At -170 mV, a potential at which a great deal of inactivation occurs, the peak current showed a direct relationship to $[\text{Na}^+]_o$, but changing $[\text{Na}^+]_o$ did not appear to affect the size of the steady state current.

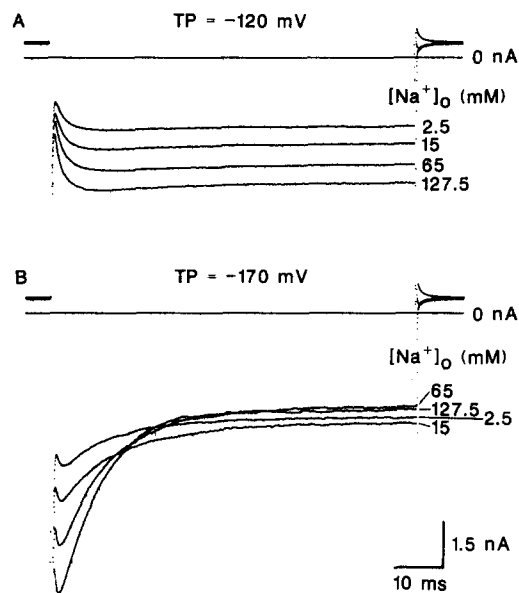


FIGURE 11. Effect of varying $[\text{Na}^+]_o$ on current elicited at -120 mV (A), where little decline of steady state current occurs, and at -170 mV (B), where a significant decline of steady state current occurs, from a cell exposed to 2.5, 15, 65, and 127.5 mM $[\text{Na}^+]_o$.

DISCUSSION

Comparison of I_{K1} with Other Inward-rectifying K^+ Currents

The results of this study suggest that inward rectification in cat ventricular myocytes resembles the inward rectification found in noncardiac preparations. Certain characteristics of the inward-rectifying K^+ current found in these myocytes resembled the background cardiac K^+ current referred to as I_{K1} . In addition, there were similarities to the inward-rectifying K^+ currents in frog skeletal muscle and tunicate and starfish egg cells. In order to better understand the properties of I_{K1} , the characteristics of the inward-rectifying K^+ current in cat ventricular myocytes were compared with those of the well-documented currents in noncardiac preparations.

Conductance. The square-root relationship between $[\text{K}^+]_o$ and conductance reported here for cat ventricular myocytes is similar to that reported for multicellular preparations of cardiac ventricular tissue in terms of the K^+ sensitivity

of total membrane conductance (Daut, 1982). It has also been defined for the K^+ sensitivities of both single-channel and whole-cell conductances of guinea pig ventricular myocytes. In the latter case, Sakmann and Trube (1984a) determined the whole-cell conductance from I - V relationships obtained using a voltage-clamp ramp. The experiments reported here extend their observations by showing that the square-root relationship applies to the peak as well as the steady state components of I_{K1} .

The inward-rectifying K^+ current in noncardiac preparations exhibits a true instantaneous component (Hagiwara et al., 1976; Hestrin, 1981; Leech and Stanfield, 1981). This is due to the fact that inward currents were elicited from a holding potential set at E_K , which activated the conductance of the membrane to K^+ . In cat ventricular myocytes, an instantaneous inward component of I_{K1} could not be detected after hyperpolarization of the membrane from a holding potential of -40 mV. This suggests that I_{K1} conductance is close to zero at this potential when $[K^+]_o$ is 5.4 mM. Therefore, the outward current measured at potentials positive to E_{K1} may actually be contaminated by some non- I_{K1} current. At higher $[K^+]_o$, g_{K1} would not be minimal at -40 mV (see Fig. 5 A) and a true instantaneous current jump would be expected from hyperpolarization to potentials negative to E_K .

The ($V_M - E_K$) relationship of the K^+ and voltage dependence of conductance corresponds well to that found in noncardiac preparations. However, this does not imply that the conductance in cat ventricular myocytes depends solely on the K^+ driving force. In starfish egg cells (Hagiwara and Yoshii, 1979) and frog skeletal muscle (Hestrin, 1981; Leech and Stanfield, 1981), changing $[K^+]_i$ affected the maximum conductance level as expected, but did not result in a shift of the relationship along the voltage axis such as occurred when $[K^+]_o$ was altered.

Changes seen in current magnitude when $[Na^+]_o$ is changed have also been reported in tunicate egg cells (Ohmori, 1978, 1980; Fukushima, 1982). Although the mechanism is not understood, Ohmori (1980) suggested a Na^+ dependence for channel activation (as well as inactivation) because of the increase in apparent channel activity seen using fluctuation analysis. Fukushima (1982), however, suggested that extracellular Na^+ may facilitate single-channel conductance. The mechanism behind the effects in isolated cat ventricular myocytes was not investigated here; however, there are several possible consequences associated with the reduction of $[Na^+]_o$, many of which could modulate or regulate the I_{K1} conductance.

Activation. The results from experiments using isolated cat ventricular myocytes are consistent with recent reports indicating that a time-dependent activation of I_{K1} occurs in guinea pig ventricle (Kurachi, 1985; Mitra et al., 1986) and canine Purkinje cells (Pennefather et al., 1987). However, there appear to be some differences in the rate and voltage dependence of inward-rectifying K^+ current activation in cat ventricular myocytes and in noncardiac preparations. In frog skeletal muscle, experiments performed at 2 – 5°C yielded time constants of activation approaching 40 – 50 ms at voltages near E_K when $[K^+]_i$ was not

altered (Hestrin, 1981; Leech and Stanfield, 1981). On the other hand, Hagiwara et al. (1976) found that the current in starfish egg cells activated at a rate 10 times slower even at higher temperatures (22–23°C). If temperature differences are accounted for by assuming a Q_{10} of 2.64 (Leech and Stanfield, 1979), the rate of activation of inward K⁺ current at potentials near E_K in ventricular myocytes and skeletal muscle appear to be similar.

As described in skeletal muscle (Leech and Stanfield, 1981) and starfish egg cell preparations (Hagiwara et al., 1976), the relationship between the activation time constant and the membrane potential at any $[K^+]_o$ is exponential. However, in cat ventricular myocytes, τ_A appears to be less steeply voltage dependent. The time constant shows a 10-fold decline for a 90-mV hyperpolarization, whereas a 10-fold decline for a 41-mV hyperpolarization is found in skeletal muscle (Leech and Stanfield, 1979). The experiments in skeletal muscle evaluated current elicited during test pulses from a holding potential set at E_K . The holding potential for all experiments presented here was -40 mV. If a difference in holding potential could explain the difference in the steepness of the voltage dependence of activation, τ_A would be expected to be more steeply voltage dependent when $[K^+]_o$ was increased because E_K would approach the holding potential. The results presented here do not support this possibility (see Fig. 6B). Even though the activation properties of I_{K1} in cat ventricular myocytes are not quantitatively identical to those of the inward-rectifying K⁺ currents in noncardiac preparations, they do appear to be qualitatively similar.

In addition, the K⁺ and voltage dependence of activation resembles that in skeletal muscle and starfish egg cells in that changing $[K^+]_o$ causes a shift in the τ_A -voltage relationship that is proportional to the change in E_K . In frog skeletal muscle preparations (Hestrin, 1981; Leech and Stanfield, 1981) and starfish egg cells (Hagiwara et al., 1976), this relationship did not shift when E_K was altered by changing $[K^+]_i$. This has been taken to suggest that there may be an extracellular K⁺-binding site that modulates the single-channel gating parameters (Hestrin, 1981).

Inactivation. I_{K1} elicited by strong hyperpolarizations in cat ventricular myocytes decreased with time. This voltage- and time-dependent inactivation resembles the inactivation of inward-rectifying K⁺ currents in noncardiac preparations. In frog skeletal muscle, the inactivation of the inward K⁺ current has been attributed to a time-dependent change in conductance caused by depletion of K⁺ from extracellular spaces as well as to time- and voltage-dependent changes in permeability (Adrian et al., 1970; Almers, 1972a, b). This conclusion appears to be true for cat ventricular cells as well. The contribution of depletion to the time-dependent decline could be slightly greater than that estimated (Fig. 8B) because the tail currents used to determine the shift in E_K were decaying inwardly. Therefore, the instantaneous current level would be more outward and the true reversal potential would be slightly more negative. However, depletion of extracellular K⁺ cannot account for the entire change in the current amplitude, especially that which results in the negative slope region of the steady state I - V relationship. An additional argument against depletion being the only

mechanism underlying the time-dependent decline is that a process that is K^+ dependent would not be expected to be particularly sensitive to changes in $[Na^+]_o$, as was shown here (Fig. 10).

In tunicate eggs, removal of extracellular Na^+ results in a linearization of the steady state $I-V$ relationship (Ohmori, 1978). Analysis of single-channel activity in this preparation has shown that the presence of a channel blocker (i.e., Na^+) is necessary to even see current fluctuations owing to channel closing (Ohmori, 1980). It has also been suggested that inactivation in skeletal muscle is at least partially due to a time- and voltage-dependent block of the inward current by extracellular Na^+ (Standen and Stanfield, 1979). In the experiments presented here, removing extracellular Na^+ affected the negative slope region of the steady state $I-V$ relationship, causing a decrease in the steepness of the voltage dependence. This finding suggests that extracellular Na^+ plays some role in the inactivation of I_{K1} .

The fact that the negative slope of the steady state $I-V$ relationship was not completely removed by reducing extracellular Na^+ to very low levels indicates that the presence of small amounts of Na^+ (~ 2.5 mM was added as NaOH to adjust the pH) permits some inactivation to occur. Experiments performed in the absence of any added Na^+ (TMA-OH was used to adjust the pH) resulted in the lack of a significant negative slope region even when the cells were hyperpolarized to -210 mV (data not shown). This, however, does not preclude the presence of an Na^+ -independent inactivation, because a decline of current from peak levels still occurred in the absence of extracellular Na^+ . This is consistent with the findings of Sakmann and Trube (1984*a, b*), who showed inactivation in single channels even when Na^+ was absent from the pipette (extracellular) solution.

Significance with Respect to Earlier Experiments in Cardiac Tissue

Much of the earlier information about I_{K1} was derived from experiments on either Purkinje fibers or ventricular tissue, mainly papillary muscles, and no significant differences between I_{K1} in the two preparations were reported. Therefore, the fact that many characteristics of I_{K1} found in ventricular myocytes correlate with what has been found or predicted in Purkinje fibers is not surprising. This includes the findings of McAllister and Noble (1966) that g_{K1} in Purkinje fibers depends on the $(V_M - E_K)$ relationship as well as $[K^+]_o$. In addition, the resting potential is in the steepest portion of the conductance-voltage relationship, so that g_{K1} increases at potentials negative to E_K and decreases at potentials positive to E_K .

The first direct evidence that changes in g_{K1} are not instantaneous was demonstrated by Carmeliet (1982) in sheep Purkinje fibers. He found that, under K^+ - and Na^+ -free conditions, the fiber could undergo time-dependent changes in conductance that he interpreted as an extracellular K^+ -dependent activation. This is consistent with what was found in isolated ventricular myocytes. An activating component of I_{K1} was seen when membrane potential was changed to a voltage where conductance was expected to increase. For example, in the protocol used to describe steady state inactivation (see Fig. 9), when a conditioning

prepulse to -90 mV was given, current elicited during the subsequent test pulse to -155 mV showed an activating component. However, with a prepulse to -140 mV, the subsequent test pulse resulted only in an instantaneous jump followed by inactivation. Under these conditions ($[K^+]_o = 5.4$ mM), going from -90 to -155 mV would be expected to cause an increase in the conductance (see Fig. 5 A). However, going from -140 to -155 mV would cause little if any change in conductance, consistent with the absence of a time-dependent increase or activation of the current. Hyperpolarizing pulses from potentials at which conductance is already maximal only result in an increase in the amount of current inactivated. On the other hand, depolarizing pulses between potentials where conductance is already maximal result only in the apparent recovery of inactivated current. This would explain why a test pulse to -155 mV, following a prepulse to -190 mV, results in a slowly activating current without inactivation.

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