BACKGROUND K^+ CURRENT IN ISOLATED CANINE CARDIAC PURKINJE MYOCYTES

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ABSTRACT The current–voltage (I-V) relation of the background current, I_{K1}, was studied in isolated canine cardiac Purkinje myocytes using the whole-cell, patch-clamp technique. Since Ba^{2+} and Cs^+ block I_{K1}, these cations were used to separate the I-V relation of I_{K1} from that of the whole cell. The I-V relation of I_{K1} was measured as the difference between the I-V relations of the cell in normal Tyrode (control solution) and in the presence of either Ba^{2+} (1 mM) or Cs^+ (10 mM). Our results indicate that I_{K1} is an inwardly rectifying K^+ current whose conductance depends on extracellular potassium concentration. In different $[K^+]_0$'s the I-V relations of I_{K1} exhibit crossover. In addition the I-V relation of I_{K1} contains a region of negative slope (even when that of the whole cell does not). We also examined the relationship between the resting potential of the myocyte, V_m, and $[K^+]_0$ and found that it exhibits the characteristic anomalous behavior first reported in Purkinje strands (Weidmann, S., 1956, Elektrophysiologie der Herzmuskelfaser, Med. Verlag H. Huber), where lowering $[K^+]_0$ below 4 mM results in a depolarization.

INTRODUCTION

Several studies on cardiac Purkinje fibers have characterized the time-independent background current (I_{K1}) as an inwardly rectifying K^+ current with a current–voltage (I-V) relation that exhibits external K^+ dependence and crossover (Noble, 1965; Haas and Kern, 1966; Isenberg, 1976; Carmeliet, 1982; DiFrancesco et al., 1984). There have generally been two approaches to these types of studies. In the first, the I-V relation of the total membrane current for potentials negative to ~ -50 mV is taken to be a good estimate of the I-V relation of I_{K1}, since at negative potentials the K^+ current dominates. The second approach involves the use of blockers such as Ba^{2+} or Cs^+ to block I_{K1}. The I-V relation of I_{K1} is then taken as the difference between the current recorded in the presence and absence of the blocker. The problem with either of these approaches in the Purkinje strand is that for reasons outlined below, the interpretation of results is ambiguous.

In the Purkinje strand, significant K^+ accumulation and depletion in the narrow intracellular clefts occurs during voltage-clamp steps or action potentials. Due to these $[K^+]$ fluctuations, cleft K^+ concentration can differ at times from bulk $[K^+]_0$ (Baumgarten and Isenberg, 1977; Cohen and Kline, 1982; Kline and Cohen, 1984). As a consequence, the cell membrane surfaces that face the clefts see extracellular K^+ concentrations that are inhomogeneous and different from $[K^+]_0$. Furthermore, due to cleft K^+ accumulation and depletion, time-dependent K^+ accumulation and depletion currents can arise within the Purkinje strand. Normally in the presence of a blocker of I_{K1}, for a single bulk K^+ concentration, cleft K^+ concentration differs in the presence and absence of the blocker.

A consequence of these difficulties with the measurement of I_{K1} in the Purkinje strand is that one cannot be certain if the previously reported (Isenberg, 1976; DiFrancesco et al., 1984) negative slope in the I-V relation of I_{K1} is a property of an individual component of total membrane current or due to an interplay between the different components and the strand geometry. Hence it is important that results obtained from strands be confirmed by studies from single cell preparations where the problems of extracellular K^+ accumulation or depletion are nearly eliminated (Callewaert et al., 1984; Mathias et al., 1985). The purpose of this study was to examine the properties of I_{K1} in canine Purkinje myocytes.

In our experiments we use acutely dissociated Purkinje myocytes. As will be shown in the results, we find that I_{K1} is an inwardly rectifying K^+ current that exhibits external K^+ dependence and crossover as in Purkinje strands. Further, it exhibits a region of negative slope, indicating that negative slope is a property of a pharmacologically identifiable component of total membrane current.

We also examined the relationship between the resting potential of Purkinje myocytes and extracellular K^+ concentration for $[K^+]_0$'s ranging from 1 to 64 mM. Our results indicate that this relationship is anomalous, similar to that described by Weidmann (1956) in Purkinje fibers. The resting potential closely follows predicted values of E_K for $[K^+]_0 \geq 4$ mM but deviates sharply to more positive values when $[K^+]_0$ is reduced below 4 mM.

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METHODS

Acutely isolated canine Purkinje myocytes (for dissociation procedure see Gintant et al., 1985; Cohen et al., 1987) were voltage clamped using the whole-cell, voltage-clamp technique. The patch pipettes contained (in millimolar) 140 KCl, 10 NaCl, 2 $MgCl_2$, 10 dextrose, 1 EGTA, and 5 Hepes, and had resistances of 1–3 MΩ in Tyrode solution. In later experiments, the patch pipette contained 117 mM KCl, 11 mM EGTA, 1 mM $CaCl_2$, and 10 mM Hepes, instead of 140 mM KCl, 1 mM EGTA, and 5 mM Hepes. (The pH was adjusted to 7.4 with 23 mM KOH.) Results obtained using this solution were the same as those obtained from the first solution. The solution flowing in the experimental chamber was a gassed (95% O_2–5% CO_2) Tyrode solution containing (in millimolar) 140 NaCl, 2 $CaCl_2$, 12 $NaHCO_3$, 0.4 NaH_2PO_4, 1.6 $MgCl_2$, 10 dextrose, 25 taurine, 5 B-hydroxybutyric acid, and 5 Na-pyruvate and different K^+ concentrations depending on experimental protocol. "Normal" Tyrode solution contained 8 mM KCl. (8 mM K^+ rather than lower K^+ concentrations was used to increase g_{K1} and to reduce the negative potentials to which the cells had to be routinely polarized.) When dictated by the experimental protocol, 1 mM $BaCl_2$ or 10 mM CsCl was added to the Tyrode solution. A temperature-controlled (±1°C) experimental chamber maintained a temperature of 35.5 ± 1°C during all experiments (Datyner et al., 1985).

A switched (9–12 KHz) single electrode voltage clamp (Axoclamp-2; Axon Instruments, Inc., Burlingame, CA) was used. The resistance of the patch pipette sometimes increased (to 3–8 MΩ) on sealing to the cell membrane, but we were often able to reduce it by applying small continuous positive or negative pressures. The advantage of using the switched single-electrode voltage clamp, over the continuous single-electrode voltage-clamp configuration, is that electrode resistance changes do not introduce errors in the measured voltage, since current is not passed while voltage is measured. The data were recorded on an FM tape recorder (Hewlett-Packard Co., Palo Alto, CA; 3964*a*, 1⅞ in./s 600-Hz bandwidth) for later display and analysis.

To obtain the slope conductance of I_{K1} at its reversal potential the data points in the vicinity (±10 mV) of V_{rev} were fit using least squares with either a second- or third-order polynomial. The derivative of the polynomial at V_{rev} was used as a measure of the slope conductance of the I-V relation at V_{rev}.

RESULTS

To be measured, I_{K1} must be separated from the pacemaker current, I_f, and the inward background and pump currents, which are also present in the same voltage range. This was accomplished by using either Ba^{2+} or Cs^+ to block I_{K1}. 1 mM Ba^{2+} totally blocks I_{K1} with only small effects on I_f (concentrations of Ba^{2+} above 1 mM block I_f more substantially [Cohen et al., 1983]). In contrast, 10 mM Cs^+ blocks both I_{K1} and I_f (Isenberg, 1976). However, since a large proportion of our cells exhibited no pacemaker current, we were able to use Cs^+ to selectively block I_{K1}. Since most of our experiments were performed at $[K^+]_o$'s > 4 mM, the effects of Cs^+ on the Na^+/K^+ pump are small (the K_M in these cells is 0.8 mM K^+ [Cohen et al., 1987]. For the experiments in 1 mM $[K^+]_o$, results in Cs^+ were similar to those in Ba^{2+}.

Fig. 1, *A–C* illustrates the total membrane current recorded from three different cells in response to 5-s-long hyperpolarizing voltage steps from a holding potential of −50 mV. Fig. 1 *A, bottom,* shows the current recorded in the first 100 ms in response to 5-s-long hyperpolarizing voltage steps from −50 mV to −70, −75, −79, and −83 mV (shown in Fig. 1 *A, top*). It is apparent on this time scale that there is little inactivation of the current. We made this observation consistently in all our experiments. As seen in Fig. 1, *B* and *C,* inactivation is largely absent even on the longer time scales. The effect of Cs^+ is shown in Fig. 1 *B*. The protocol was as follows. With normal 8 mM K^+ Tyrode as the bath solution, the cell was voltage clamped at −50 mV and 5-s-long voltage steps to −40, −20, −80, and −85 mV were applied. The current traces are shown in the left-hand side of Fig. 1 *B*. After this, 10 mM CsCl was added to the Tyrode solution. The right-hand side of Fig. 1 *B* illustrates the current traces recorded in Cs^+; Cs^+ blocks the inward rectifying component of the total membrane current. Fig. 1 *C* is similar to *B* except that 1 mM $BaCl_2$, instead of 10 mM CsCl, was used to block I_{K1}.

The I-V relation of the Purkinje myocyte was first obtained in the control solution and then in the presence of either 1 mM Ba^{2+} or 10 mM Cs^+. The difference between the control and the test I-V relations was used to estimate the I-V relation of I_{K1}.

Fig. 2 *A* shows results obtained from an experiment on a cell that did not exhibit pacemaker current. Here, 10 mM CsCl was used to block I_{K1}. 20 mM CsCl yielded essentially the same results, suggesting that at these concentrations the Cs^+ block of I_{K1} was total and the negative slope was not due to relief of this block at positive potentials. Since I_{K1} does not inactivate appreciably in the potential range we studied (see Fig. 1), the steady-state I-V relations are similar to the quasi-instantaneous (t = 25 ms) ones. In Fig. 2 *A* the myocyte was held at −50 mV, and 5-s-long hyperpolarizing and depolarizing test pulses were applied. The I-V relations were measured first in normal 8 mM K^+ Tyrode and then in the presence of 10 mM Cs^+. In the presence of Cs^+, at potentials negative to −40 mV an inwardly rectifying component of membrane current is blocked. The difference I-V relation, I_{K1}, illustrated in Fig. 2 *B,* shows inward rectification and negative slope. The magnitude of I_{K1}, is close to zero at potentials positive to −40 mV. Fig. 2, *C* and *D* illustrates results from a similar experiment; in this example 1 mM Ba^{2+} was used to block I_{K1}. Again, the difference I-V relation of I_{K1} exhibits inward rectification and negative slope. However, at potentials positive to −40 mV, Ba^{2+} also partly blocks I_x (Gintant et al., 1985) and may affect other membrane currents. For example, mixtures of Ba^{2+} and Ca^{2+} alter the magnitude of current though the slow inward channel (Hess et al., 1983). Cs^+ seemed to have only small effects on I_x. Fig. 2, *B* and *D* indicates that the reversal potential of I_{K1} is ~−75 mV in 8 mM $[K^+]_o$. Given the 140 mM K^+ in the pipette and $[K^+]_o$ = 8 mM, E_K is −76 mV, suggesting that I_{K1} is almost exclusively carried by K^+ ions. An interesting observation in all our experiments of this type is that whereas the total membrane I-V relation did not always exhibit negative slope, the I-V relation of I_{K1} always contained such a region (see Fig. 2).

Next, we tried to determine the dependence of I_{K1} on

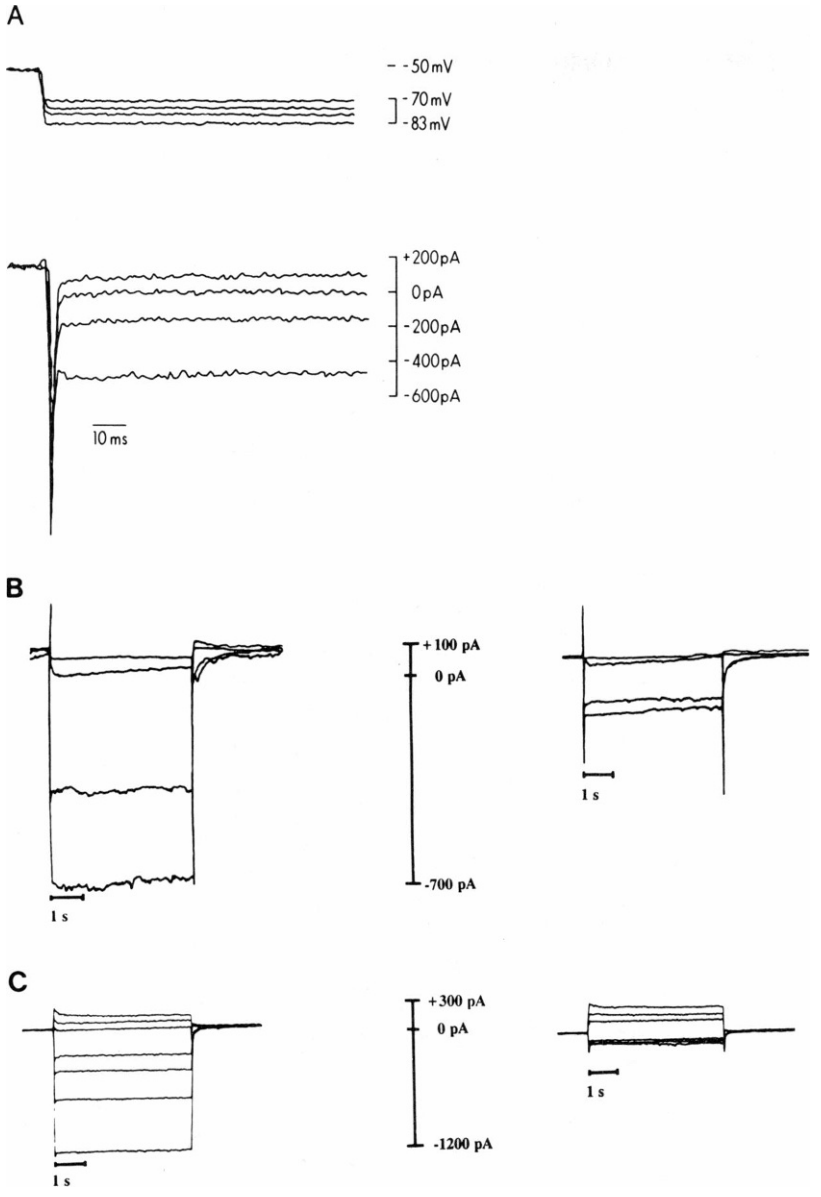

FIGURE 1 Time course of I_{K1} and effects of Cs^+ (10 mM) and Ba^{2+} (1 mM). (*A*) *Top*, initial 100 ms of 5-s voltage-clamp pulses from a holding potential of −50 mV, to −70, −75, −79, and −83 mV (the noise on the voltage trace is created on playback from the FM recorder). *Bottom*, current in response to these voltage-clamp pulses in 8 mM K^+ Tyrode solution. (*B*) *Left*, current in response to 5-s long voltage-clamp pulses from a holding potential of −50 mV to −40, −20, −80, and −85 mV in 8 mM K^+ Tyrode solution. *Right*, currents after the addition of 10 mM Cs^+, for the same voltage-clamp pulses. (*C*) Same protocol as *B*. 1 mM Ba^{2+} was used instead of Cs^+ to block I_{K1}. Holding potential: −50 mV, voltage-clamp pulses to −20, −10, 0, −74, −77, −80, and −83 mV.

$[K^+]_0$. Previous reports from single ventricular cells indicate that I_{K1} has a conductance proportional approximately to the square root of $[K^+]_0$ and that the I-V relations in different $[K^+]_0$'s exhibit crossover (Sakmann and Trube, 1984). To determine if this could be seen in our Purkinje myocytes, the I-V relation of the myocyte was first obtained in two control Tyrode solutions containing different K^+ concentrations. 10 mM Cs^+ or 1 mM Ba^{2+} was then added to each solution and the I-V relations measured again. Fig. 3 illustrates the crossover in the difference I-V relations from such an experiment using 4 mM K^+ and 12 mM K^+ extracellular solutions and 10 mM Cs^+ as the I_{K1} blocker. To determine the $[K^+]_0$ dependence of the slope conductance of I_{K1} (g_{K1}) at the reversal potential, we fit the data in the vicinity of V_{rev} as described in the Methods section. From this analysis g_{K1} is 119 ± 10 $\mu S/\mu F$ (mean ± SEM, $n = 3$) for $[K^+]_0$ = 12 mM, 114 ± 15 $\mu S/\mu F$ ($n = 6$) for $[K^+]_0$ = 8 mM, and 91 ± 23 $\mu S/\mu F$ ($n = 3$) for $[K^+]_0$ = 4 mM. These numbers are consistent with a g_{K1} proportional to the cube root of $[K^+]_0$ in this range of $[K^+]_0$'s. An accurate determination of g_{K1} at low $[K^+]_0$ concentrations was difficult since at these concentrations, the magnitude of I_{K1} is very small.

The selectivity of I_{K1} was examined next, by looking at the zero current potential (V_{rev}) of the difference current. We find that the V_{rev} of I_{K1} is within a few millivolts of E_K when $[K^+]_0 > 4$ mM; for $[K^+]_0$ = 12 mM, average V_{rev} ($n = 3$, ±SEM) is −64 ± 2.4 mV (E_K = − 65 mV), for $[K^+]_0$ = 8 mM, average V_{rev} ($n = 9$, ±SEM) is −74 ± 1.1 mV (E_K = −76 mV), and for $[K^+]_0$ = 4 mM, average V_{rev} ($n = 5$, ±SEM) is −91 ± 1.7 mV (E_K = 94 mV). In 1 mM $[K^+]_0$ average V_{rev} ($n = 3$, ±SEM) is −116 ± 1.7 mV (E_K = −131 mV). To determine if this apparently positive value of V_{rev} in 1 mM $[K^+]_0$ was due to a significant

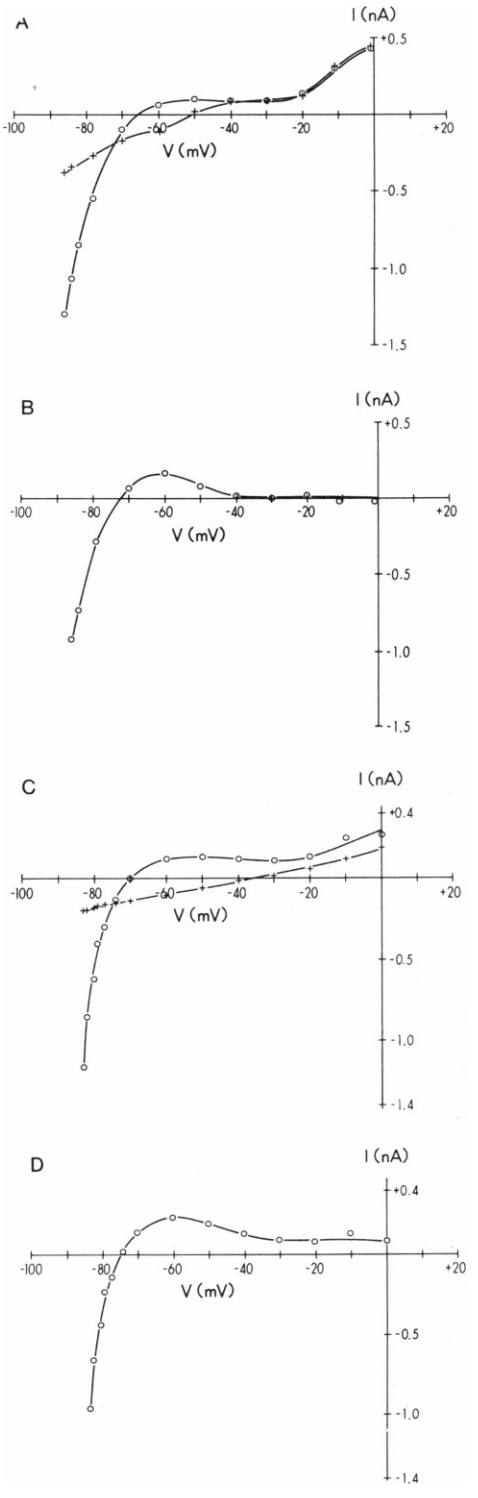

FIGURE 2 Effect of Cs^+ (10 mM) and Ba^{2+} on steady-state membrane I-V relations. Effect of Cs^+: (*A*) Holding potential was −50 mV, voltage-clamp pulses of 5-s duration were applied in positive and negative directions. (O) 8 mM K^+ Tyrode solution, (+) 8 mM K^+ Tyrode solution + 10 mM Cs^+. Each point is the current measured just before the end of the 5-s voltage-clamp pulse. (*B*) Difference I-V relation; each point is the difference in current between control (8 mM K^+ Tyrode) solution and test (8 mM K^+ Tyrode + Cs^+) solution. Effect of Ba^{2+}: (*C*) Same protocol as in *A*. (O) 8 mM K^+ Tyrode solution, (+) addition of 1 mM Ba^{2+}. (*D*) Difference I-V relation (control-barium).

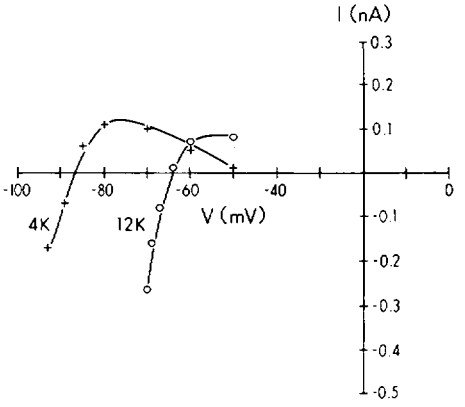

FIGURE 3 Effect of $[K^+]_0$ on steady-state I-V relation of I_{K1}. Voltage-clamp pulses of 5-s duration were applied in positive and negative directions from a holding potential of −50 mV. Each point on each trace is the difference between currents recorded in control solution and in the presence of 10 mM Cs^+. (+) 4 mM K^+ Tyrode solution, (O) 12 mM K^+ Tyrode solution.

decrease in selectivity, we used the Goldman–Hodgkin–Katz equation (Hodgkin and Katz, 1949) to calculate the P_K/P_{Na} ratio for I_{K1}, at the four different $[K^+]_0$'s. The inherent assumption here is that the I_{K1} channel is permeant only to monovalent cations. Our results indicate that for $[K^+]_0$'s from 1 to 12 mM, P_K/P_{Na} was >150, suggesting that even at low $[K^+]_0$'s, I_{K1} is still highly selective to K^+ ions. Fig. 4 illustrates the data, the V_{rev} versus $[K^+]_0$ relationship expected for P_K/P_{Na} ratios of 100 and 200 and the relationship for a Nernstian K^+ electrode. From the figure, it is apparent that the data points for 1, 4, 8, and 12 mM $[K^+]_0$ all lie along the line predicted for a P_K/P_{Na} of 200:1 with a slope of near −61 mV/10-fold change in $[K^+]_0$.

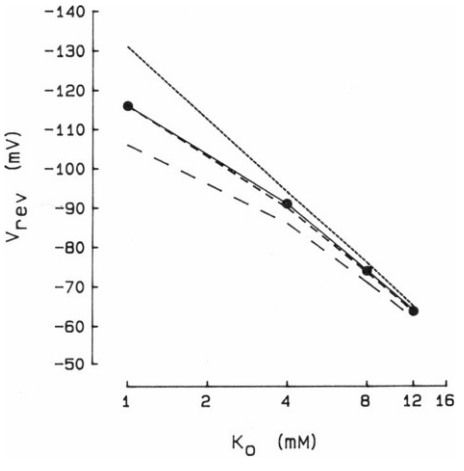

FIGURE 4 Semilogarithmic plot of the relationship of the reversal potential (V_{rev}) of I_{K1} with $[K^+]_0$. (●) Data, each point is V_{rev} averaged over a number of experiments (12 mM K^+, n = 3, 8 mM K^+, n = 9, 4 mM K^+, n = 5, 1 mM K^+, n = 5. In all cases the standard error was ≤2.4 mV); (— — —) plot expected if a P_K/P_{Na} ratio of I_{K1} = 100; (- - -) plot expected if a P_K/P_{Na} ratio of I_{K1} is 200; (· · · · · ·) Nernstian plot.

It has been known for some time that in Purkinje fibers the relationship between V_m and $[K^+]_0$ is anomalous; at low $[K^+]_0$'s, V_m decreases (Weidmann, 1956). This effect is attributed to the $[K^+]_0$ dependence of I_{K1} (Baumgarten and Fozzard, 1986). Since g_{K1} is dependent on K_0, at low $[K^+]_0$'s the resting Na^+ conductance relatively increases in importance, thereby bringing V_m closer to E_{Na} and to more positive levels. To see if the same anomalous relationship exists in Purkinje myocytes we measured the resting potential of the myocyte in $[K^+]_0$'s ranging from 1 to 64 mM. The results are illustrated in Fig. 5, *A* and *B*. Fig. 5 *A* is a plot of the V_m versus $[K^+]_0$ relationship of a single cell and *B* illustrates the average values of V_m obtained for different $[K^+]_0$'s from a number of experiments. $[K^+]_0$ is plotted on the abscissa using semilog coordinates and V_m on the ordinate. As seen, when $[K^+]_0$ is 4 mM or higher, V_m is close to E_K; however, at $[K^+]_0$ below 4 mM the resting potential of the myocyte is either at a resting potential more positive to E_K or is spontaneously active.

DISCUSSION

Results from the experiments presented above suggest that also in dissociated canine Purkinje myocytes, I_{K1} is an inwardly rectifying current whose conductance increases with extracellular K^+ concentration and whose I-V relation exhibits crossover. We find the permeability ratio P_K/P_{Na} for I_{K1} is >150 for $[K^+]_0$'s ranging from 1 to 12 mM, suggesting that this current is carried mostly by K^+ ions.

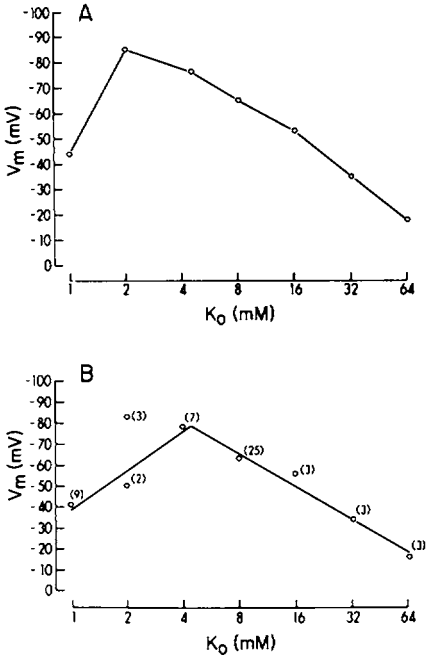

FIGURE 5 Semilogarithmic plot of the relationship of the resting potential (V_m) with $[K^+]_0$. (*A*) V_m versus $[K^+]_0$ relationship of a single cell. The cell was exposed successively to 8, 16, 32, 64, 4, 2, and 1 mM $[K^+]_0$ and the corresponding resting potential measured. (*B*) Average V_m versus $[K^+]_0$ relation. Each point is the V_m obtained averaged for a number of cells. (64, 32, and 16 mM $[K^+]_0$, $n = 3$, 8 mM $[K^+]_0$, $n = 25$, 4 mM $[K^+]_0$, $n = 5$, 2 mM $[K^+]_0$, $n = 5$ and 1 mM $[K^+]_0$, $n = 9$.) In all cases the standard error was <1.6 mV. In 2 mM $[K^+]_0$, when V_m was −84 mV, the cells were spontaneously active.

Sakmann and Trube (1984), in their single channel studies on guinea pig ventricular myocytes, found that the inwardly rectifying region of the single channel I-V relation of I_{K1} could be well fit by a constant conductance. We did not find this to be the case for the macroscopic I_{K1} in Purkinje myocytes, suggesting that an increase in open time at negative potentials is expected (Kurachi, 1985). To obtain the slope of the I_{K1} I-V relation the data points in the vicinity (±10 mV) of V_{rev} were fit by a second- or third-order polynomial (we have no theoretical basis for this). However, at potentials more than 10 mV from V_{rev} even this approach did not achieve a good fit. Cleeman and Morad (1979) have developed a model for the I_{K1} I-V relation from frog ventricle. We tested their model on our data and found that for a suitable I_{max} (maximum outward current) the I-V relation generated by the model was, again, not sufficiently steep to fit our data in either the inwardly rectifying or the negative slope regions.

In addition to exhibiting extracellular K^+ dependence and crossover, the I-V relation of I_{K1} contains a region of negative slope. This has previously been observed in radioactive K flux studies (Haas and Kern, 1966; Vereecke et al., 1980), and therefore argues against a voltage-dependent action of Cs^+. Further evidence is provided by the analysis of the kinetics of I_{K1} (in whole cell mode), which indicates a low open probability at these potentials positive to E_K (Pennefather, P., N. K. Mulrine, D. DiFrancesco, and I. S. Cohen, unpublished observations). An important consequence of this negative slope is that it makes I_{K1} virtually absent at plateau potentials. Thus, in Purkinje myocytes, I_{K1} seems to be active predominantly in the diastolic potential range and does not significantly control the cardiac action potential duration. This observation has particular physiological significance, when one considers the geometry of the Purkinje strand (Eisenberg and Cohen, 1983). In the strands, the K^+ concentration continuously fluctuates within the narrow intracellular clefts (Cohen and Kline, 1982). Since the plateau of the action potential determines in part the rate and force of contraction of the heart, it would be a potentially serious problem if the plateau was influenced by the $[K^+]$ fluctuations of a large I_{K1} in addition to those of I_x. Even in the absence of effects of I_{K1}, the action potential duration is still considerably affected by changes in $[K^+]_0$ (Weidmann, 1956). On a more cellular level, the existence of negative slope in the I-V relation of I_{K1} demonstrates that negative slope can be a characteristic of a pharmacologically separable ionic current rather than as a result of a balance of inward and outward currents. However, with the protocols we have used, we cannot determine if I_{K1} is carried through single or multiple channel types and therefore cannot identify if negative slope is a property of a

single channel type. This is a particularly relevant question since Sakmann and Trube (1984) do not see appreciable single channel currents in the outward direction in their investigation. We did not see significant inactivation of I_{K1} in the potential range that we studied, even though inactivation of I_{K1} has been reported at more hyperpolarized potentials in Purkinje strands and in both atrial and ventricular single cell preparations (DiFrancesco et al., 1984; Sakmann and Trube, 1984; Lipsius et al., 1986).

It is worthwhile to discuss the other K^+ channels that might be present in the diastolic range of potentials and their contribution to our measurements of I_{K1}. These are (*a*) the ATP-regulated K^+ channel (Noma, 1983; Trube and Hescheler, 1984), (*b*) the $[Na^+]_i$-activated K^+ channel (Kameyama et al., 1984), and (*c*) the acetylcholine (ACh)-activated K^+ channel (Sakmann et al., 1983; Carmeliet and Mubagwa, 1986*a–c*).

For the ATP-regulated K^+ channel to open, intracellular ATP must fall below 1 mM (Noma, 1983). Normal $[ATP]_i$ in cardiac cells is reported to be between 3 and 4 mM (Khairallah and Mommaerts, 1953). Our pipette solution did not contain ATP, although the Na^+/K^+ pump is seen to be working normally in the cells as measured both by intracellular microelectrodes (Cohen et al., 1987) and by the whole-cell, patch-clamp technique (unpublished observations). However, this does not exclude the possibility that $[ATP]_i$ in the cells has fallen below 1 mM and that the $[ATP]_i$-dependent current does contribute to our measurements of I_{K1}.

The $[Na^+]_i$-activated K^+ channel is activated by $[NA^+]_i > 20$ mM (Kameyama et al., 1984). Our patch pipette contained 15 mM Na^+, which with an activity coefficient of 0.75 yields an expected a^i_{Na} of 11.2 mM. (In a few later experiments, the patch pipette contained 20 mM Na^+, which yields an a^i_{Na} of 15 mM.) The measured intracellular activity of Na^+ in Purkinje fibers is 6–8 mM (Ellis, 1977; Eisner et al., 1981; Lee and Dagostino, 1982). Thus it is extremely unlikely that our a^i_{Na} was >20 mM. We therefore believe that this current does not significantly contribute to our measurements.

The situation with the ACh-activated K^+ current is more complicated. ACh-induced current is present in Purkinje strands (Carmeliet and Ramon, 1980; Carmeliet and Mubagwa, 1986*a–c*). Although these results establish its presence and macroscopic characteristics in the presence of ACh, single channel studies need to be undertaken, to determine if the channel is spontaneously active in the absence of ACh. Previous single channel studies of this current in nodal cells indicate that the ACh-activated K^+ channel has characteristics similar to the resting K^+ channels in nodal cells, but different to the resting K^+ channels in ventricular and atrial cells (Sakmann et al., 1983). If the ACh-activated K^+ channel is spontaneously active in the absence of ACh, then, it would contribute to our measurements of I_{K1}, since it is also blocked by Ba^{2+} (Carmeliet and Mubagwa, 1986*b*). In this circumstance it could be a significant component of background K^+ permeability.

The relationship between $[K^+]_0$ and V_m of the Purkinje myocyte is anomalous, similar to that first reported by Weidmann in Purkinje fibers. For extracellular $[K^+]_0$'s of 4 mM or more V_m closely follows E_K, but for $[K^+]_0$ <2–4 mM, V_m deviates sharply and assumes more positive levels. As mentioned above, this deviation is due to a reduction in g_{K1}. In 8 mM $[K^+]_0$, V_m is ~10 mV positive to E_K, which is very similar to the 8.4 mV predicted from previous studies of the pump current in the same preparation (Cohen et al., 1987).

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REFERENCES

Baumgarten, C. M., and G. Isenberg. 1977. Depletion and accumulation of potassium in the extracellular clefts in cardiac Purkinje fibers during voltage clamp hyperpolarization and depolarization. *Pfluegers Arch. Eur. J. Physiol.* 360:19–31.

Baumgarten, C. M., and H. A. Fozzard. 1986. The resting and pacemaker potentials. *In* The Heart and Cardiovascular System. H. A. Fozzard, E. Haber, R. B. Jennings, and A. M. Katz, editors. Raven Press, New York.

Callewaert G., E. Carmeliet, and J. Vereecke. 1984. Single cardiac Purkinje cells: general electrophysiology and voltage-clamp analysis of the pace-maker current. *J. Physiol. (Lond.).* 349:643–661.

Carmeliet, E. 1982. Induction and removal of inward-going rectification in sheep cardiac Purkinje fibers. *J. Physiol. (Lond.).* 327:285–308.

Carmeliet, E., and K. Mubagwa. 1986*a*. Changes by acetylcholine of membrane currents in rabbit cardiac Purkinje fibers. *J. Physiol. (Lond.).* 371:201–217.

Carmeliet, E., and K. Mubagwa. 1986*b*. Characterization of the acetylcholine-induced potassium current in rabbit cardiac Purkinje fibers. *J. Physiol. (Lond.).* 371:219–237.

Carmeliet, E., and K. Mubagwa. 1986*c*. Desensitization of the acetylcholine-induced increase of potassium conductance in rabbit cardiac Purkinje fibers. *J. Physiol. (Lond.).* 371:239–255.

Carmeliet, E., and J. Ramon. 1980. Effects of acetylcholine on time-independent currents in sheep cardiac Purkinje fibers. *Pfluegers Arch. Eur. J. Physiol.* 387:207–216.

Cleeman, L., and M. Morad. 1979. Potassium currents in frog ventricular muscle: evidence from voltage clamp currents and extracellular K accumulation. *J. Physiol. (Lond.).* 286:113–143.

Cohen, I. S., N. B. Datyner, G. A. Gintant, N. K. Mulrine, and P. Pennefather. 1987. Properties of an electrogenic sodium/potassium pump in isolated canine Purkinje myocytes. *J. Physiol. (Lond.).* 383:251–267.

Cohen, I. S., R. T. Falk, and N. K. Mulrine. 1983. Actions of barium and rubidium on membrane currents in canine Purkinje fibers. *J. Physiol. (Lond.).* 338:589–612.

Cohen, I. S., and R. Kline. 1982. K^+ fluctuations in the extracellular spaces of cardiac muscle. Evidence from the voltage clamp and extracellular K^+ selective microelectrodes. *Circ. Res.* 50:1–16.

Datyner, N. B., G. A. Gintant, and I. S. Cohen. 1985. Versatile

temperature controlled tissue bath for studies of isolated cells using an inverted microscope. *Pfluegers Arch. Eur. J. Physiol.* 403:318–323.

DiFrancesco, D., A. Ferroni, and S. Visentin. 1984. Barium-induced blockade of the inward rectifier in calf Purkinje fibers. *Pfluegers Arch. Eur. J. Physiol.* 402:446–453.

Eisenberg, B., and I. S. Cohen. 1983. The ultrastructure of the cardiac Purkinje strand in the dog: a morphometric analysis. *Proc. R. Soc. Lond. B. Biol. Sci.* 217:191–193.

Eisner, D. A., W. J. Lederer, and R. D. Vaughan-Jones. 1981. The dependence of sodium pumping and tension on intracellular sodium activity in voltage-clamped sheep Purkinje fibers. *J. Physiol. (Lond.).* 317:163–187.

Ellis, D. 1977. The effects of external cations and ouabain on the intracellular sodium activity of sheep heart Purkinje fibres. *J. Physiol. (Lond.).* 273:211–240.

Gintant, G. A., N. B. Datyner, and I. S. Cohen. 1985. Gating of delayed rectification in acutely isolated canine cardiac Purkinje myocytes: evidence for a single voltage-gated conductance. *Biophys. J.* 48:1059–1064.

Haas, H., and R. Kern. 1966. Potassium fluxes in voltage clamped Purkinje fibers. *Pfluegers Arch. Eur. J. Physiol.* 291:69–84.

Hess, P., K. S. Lee, and R. W. Tsien. 1983. Ion-ion interactions in the Ca channel of single heart cells. *Biophys. J.* 41(2, Pt. 2):293a. (Abstr.)

Hodgkin, A. L., and B. Katz. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol. (Lond.).* 108:37–77.

Isenberg, G. 1976. Cardiac Purkinje fibers: cesium as a tool to block inward-rectifying potassium currents. *Pfleugers Arch. Eur. J. Physiol.* 365:99–106.

Kameyama, M., M. Kakei, R. Sato, T. Shibasaki, H. Matsuda, and H. Irisawa. 1984. Intracellular Na^+ activates a K^+ channel in mammalian cardiac cells. *Nature (Lond.).* 309:354–356.

Khairallah, P. A., and W. F. H. M. Mommaerts. 1953. Nucleotide metabolism in cardiac activity. I. Methods. *Circ. Res.* 1:8–11.

Kline, R., and I. S. Cohen. 1984. Extracellular $[K^+]$ fluctuations in voltage-clamped canine cardiac Purkinje fibers. *Biophys. J.* 49:663–668.

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

Lee, C., and M. Dagostino. 1982. Effect of strophanthidin on intracellular Na ion activity and twitch tension of constantly driven canine cardiac Purkinje fibers. *Biophys. J.* 40:185–198.

Lipsius, S., J. Vereecke, and E. Carmeliet. 1986. Pacemaker current (i_f) and the inward rectifier (I_{K1}) in single right atrial myocytes from cat heart. *Biophys. J.* 49(2, Pt. 2):351a. (Abstr.)

Mathias, R., B Eisenberg, N. B. Datyner, G. A. Gintant, and I. S. Cohen. 1985. Impedence and morphology of isolated canine cardiac Purkinje myocytes: comparison with intact strand preparations. *Biophys. J.* 47(2, Pt. 2):499a. (Abstr.)

Noble, D. 1965. Electrical properties of cardiac muscle attributable to inward going (anomalous) rectification. *J. Cell. Comput. Physiol.* 66(Suppl. 2):127–136.

Noma, A. 1983. ATP-regulated K^+ channels in cardiac muscle. *Nature (Lond.).* 305:147–148.

Sakmann, B., and G. Trube. 1984. Conductance properties of single inwardly rectifying potassium channels in ventricular cells from guinea-pig heart. *J. Physiol. (Lond.).* 347:641–657.

Sakmann, B., A. Noma, and W. Trautwein. 1983. Acetylcholine activation of single muscarinic K^+ channels in isolated pacemaker cells of the mammalian heart. *Nature (Lond.).* 303:250–253.

Trube, G., and J. Hescheler. 1984. Inward-rectifying channels in isolated patches of the heart cell membrane: ATP-dependence and comparison with cell-attached patches. *Pfluegers Arch. Eur. J. Physiol.* 401:178–184.

Vereecke, J., G. Isenberg, and E. Carmeliet. 1980. K efflux through inward rectifying K channels in voltage clamped Purkinje fibers. *Pfluegers Arch. Eur. J. Physiol.* 384:207–217.

Weidmann, S. 1956. Elektrophysiologie der Herzmuskelfaser. Med. Verlag H. Huber, Bern.