Background Potassium Current Active During the Plateau of the Action Potential in Guinea Pig Ventricular Myocytes

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Background outward K⁺ currents in guinea pig ventricular myocytes were characterized over a broad range of membrane potentials, including those corresponding to the plateau of the action potential. The background current that is blocked by 1 mM Ba²⁺ (I_k,b) activates within 5 msec at positive potentials, does not inactivate, and deactivates very rapidly on repolarization. I_k,b is insensitive to Cl⁻ channel blockers, internal or external [Cl⁻], dihydropyridines, and sulfonureas. In contrast, the delayed rectifier K⁺ current (I_k) was not completely blocked even by 30 mM Ba²⁺. Ba²⁺-sensitive current density increased progressively from 0.16±0.04 pA/pF at 0 mV to 0.52±0.21 pA/pF at +80 mV (n=13, mean±SEM). The background current remains present when [K⁺]o is reduced to 0 mM, which suppresses the inward rectifier K⁺ current (I_ki). These and other features suggest that I_k,b is generated by K⁺ channels that are distinct from I_k or I_ki. The kinetics and voltage dependence of I_k,b render it capable of modulating both the height and duration of the cardiac action potential. (Circulation Research 1993;72:890–900)

KEY WORDS • patch clamp • potassium channels • action potential duration • action potential repolarization • class III antiarrhythmic agents

Action potentials in the heart are 10 to 100 times longer than those in axons or in skeletal muscle,1,2 a fact that has important consequences for excitation–contraction coupling3 and arrhythmogenesis.4 Despite decades of investigation,5 our understanding of the ionic currents that underlie the plateau continues to evolve. Initially, action potential duration was thought to be determined solely as a balance of the inward Ca²⁺ current and one or at most two outward K⁺ currents; repolarization would occur as Ca²⁺ channels inactivate and as delayed rectifier K⁺ channels turn on.6 This conceptualization has required considerable revision recently, with the recognition of two distinct types of Ca²⁺ channels,7 a variety of K⁺ channels and Cl⁻ channels, and a potentially important role of current generated by Na⁺–Ca²⁺ exchange and the Na⁺–K⁺ pump in repolarization.7–9

In the present study, we focus on the K⁺ currents that are active during the action potential plateau, with particular emphasis on the identification and macroscopic characterization of a voltage-activated background K⁺ current. Although several time-independent metabolically activated currents have been identified (cAMP-activated Cl⁻ currents,10,11 ATP-sensitive K⁺ currents [I_KATP],12 and Na⁺-activated K⁺ currents13), their influence on the action potential plateau under basal conditions is unclear. The voltage-dependent K⁺ currents in ventricular cells include the delayed rectifier K⁺ current (I_k) and the inward rectifier K⁺ current (I_ki). The inward rectifier, as its name implies, passes virtually no current at positive potentials and thus contributes little to the action potential plateau. The transient outward current (I_t), although important in cardiac tissue from a variety of species (e.g., calf Purkinje fibers14 and rabbit crista terminalis15), is absent from guinea pig ventricle, which was used in this study. I_k is thought to be of greater importance in the regulation of action potential duration: two components, fast (I_k,f) and slow (I_k,s), have been convincingly dissected pharmacologically,16 along with evidence that the activity of the faster component influences repolarization.

Even the “rapid” component, I_k,f, of the delayed rectifier current, I_k, of the heart turns on more slowly than the delayed rectifier channels found in neurons and muscle.17,18 The nerve-type voltage-dependent K⁺ channels turn on completely within 5–10 msec at positive potentials and deactivate even more rapidly on repolarization. Similar rapidly activating K⁺ channels with a conductance of 12–14 pS have been described in cardiac myocytes from chick,19,20 rat,21 and guinea pig,22 but the only extensive characterization has been at the unitary level. Nevertheless, previous reports at the whole-cell level have revealed the presence of a rapidly activating outward current, not attributable to either I_ki or I_k, at potentials corresponding to the plateau of the action potential.23–26 More recently, a new gene has been cloned from guinea pig heart,27 which encodes a K⁺ channel (K_V1.5). Although the K_V1.5 channels are...
classified as delayed rectifier channels,26 the macroscopic currents recorded in Xenopus oocytes at voltages greater than +20 mV activate and deactivate within a few milliseconds and display little or no inactivation.27 Such rapidly activating channels would essentially function as time-independent background currents on the time scale of the cardiac action potential. Unlike I,K, or I,Kr, background channels would open even during the earliest stages of the action potential; thus, they might play a role in shaping excitability distinct from that of I,K, and I,Kr.

In the present study, we dissect out the background plateau K⁺ current activated at depolarized potentials and identify its probable contribution to cardiac excitation. The characteristics of this current are such that it appears to represent the macroscopic correlate of the background K⁺ channel that Yue and Marban22 called I,Kp (the subscript "p" stands for plateau, persistent, and potential sensitive).

Materials and Methods

Cell Isolation, Bathing Solutions, and Pipette Solutions

Guinea pig ventricular myocytes were enzymatically dispersed into a high K⁺ solution containing (mM) potassium glutamate 120, KCl 25, MgCl₂ 1, HEPES 10 (pH 7.4 with NaOH), K₂H₇EGTA 0.1, and dextrose 10, as detailed elsewhere.22 The isolated cells were then placed into a perfusion bath located on the stage of an inverted microscope and superfused with various HEPES-based external solutions. The most commonly used recording solution contained (mM) NaCl 140, KCl 5, HEPES 10, MgO₄H₂ 1, Ca(OH)₂ 2, and dextrose 10 (pH 7.4). This recording solution was changed as required for the various protocols used in this study, as indicated below. In a number of experiments, the Cl⁻ was replaced by aspartate to eliminate any Cl⁻ currents. A number of experiments were performed in the absence of extracellular K⁺ to eliminate I,K. All experiments were performed at room temperature (20–22°C) to allow clear separation of I,K, which turns on very slowly at this temperature.29 A variety of pharmacological agents were added as required to isolate and identify the K⁺ current activated at membrane potentials corresponding to the plateau of the action potential. These chemical agents included the following: nitrendipine (Miles Laboratories, West Haven, Conn.) to block L-type Ca²⁺ current, glibenclamide (Sigma Chemical Co., St. Louis, Mo.) to block I,KATP, Ba⁺⁺ to block I,K and I,Kr, and 9-anthracencarboxylic acid (9-AC, Aldrich Chemical Co., Milwaukee, Wis.) to block Cl⁻ current.

Electrophysiological Recording Methods

Macroscopic transmembrane currents were recorded using the whole-cell patch-clamp configuration.30 The microelectrodes were fabricated (model P-87, Sutter Instrument Co., Novato, Calif.) from thin-walled boro-silicate glass (1.5-mm diameter, World Precision Instruments, Sarasota, Fla.) with a final resistance of 2–5 MΩ after fire-polishing (model MF-83, Narishige, Tokyo). The pipettes were placed over a thin 3 M KCl agar bridge that contained a Ag/AgCl wire. The intracellular solution contained (mM) KCl 120, NaCl 5, MgCl₂ 1, HEPES 10 (pH 7.1 with KOH), Mg-ATP 2.5, and EGTA 2. In experiments designed to eliminate Cl⁻ currents, the Cl⁻ was replaced by aspartate. Previous reports have demonstrated that aspartate does not significantly permeate Cl⁻ channels.10,11 A Ag/AgCl wire encased in a thin 3 M KCl agar bridge was placed in the bath and used as the ground reference electrode. The amount of series resistance and cell capacity compensation varied from cell to cell. In general, 40–80% of the series resistance was compensated. For the recording of single-channel currents, the methods were as described previously.22

Unless otherwise indicated, currents elicited in response to steps to various test potentials (from −100 to 70 mV) for a duration of 170 msec were recorded from a holding potential of −40 mV. Tail currents in response to repolarization to −40 mV for 20 msec were also recorded. This voltage paradigm was used to construct current–voltage relations under a variety of ionic and pharmacological conditions as discussed below and also enabled us to assess the activation and deactivation kinetics of the current. The choice of a holding potential of −40 mV facilitates comparison with previous work on I,K, inactivates Na⁺ and T-type Ca²⁺ current, and has the additional virtue that L-type Ca²⁺ current both runs down more rapidly31 and is blocked more effectively by nitrendipine at depolarized voltages.32

Data Acquisition and Electronic Circuitry

Pipettes were mounted onto the input headstage of a patch-clamp amplifier (Axopatch-1D, Axon Instruments, Foster City, Calif.). To evaluate the effect of leak currents, seal resistances were measured in the cell-attached configuration before patch rupture using 20-mV hyperpolarizing voltage pulses.33 Seals with resistances less than 10 GΩ were rejected; typically, the seal resistance was greater than 20 GΩ. Thus, the leak current is expected to be less than 1 pA per 10-mV deviation from 0 mV. Leaks of this size have been shown to have an insignificant effect on the action potential profile.33 Once electrical access into the cell was attained and capacity and series resistance compensation was achieved, appropriate command voltages were applied through the use of a personal computer equipped with communications hardware (TL-1-125, Axon Instruments) and custom-written software. The membrane currents were stored in the computer for later analysis.

Results

Background Currents in Ventricular Cells

Figure 1A shows the currents evoked by depolarization (left recordings) or hyperpolarization (right recordings) from a holding potential of −40 mV before (●) and after (□) the addition of nitrendipine. The current amplitude measured 160 msec after the beginning of the test pulse and plotted in Figure 1B shows the N shape typical of heart cells. After blockade of L-type Ca²⁺ currents by the application of 10 μM nitrendipine, the inward current evoked by depolarization is entirely absent. In the presence of nitrendipine, panel A exhibits a small time-independent outward current during the step to +30 mV; panel B shows that the magnitude of this component increases progressively with depolariza-
Ionic Identity of Internal Ground Pots since extracellular currents on our pipette current Cl- and recordings during subsequent data is small from 13, and is in the millimolar range, as it is in our pipette solutions.34

Figure 2 further examines the magnitude and voltage dependence of the background current. To remove interference from L-type Ca2+ channels, this and all subsequent figures show results obtained in the presence of 10 μM nitrendipine. Panel A shows typical current recordings during depolarizing steps. The current is always outward at positive potentials and exhibits little time dependence. Of note, there are no clear tail currents on repolarization to −40 mV. Figure 2B shows pooled data for the density of the background current from 13 cells. The density averages approximately 0.5 pA/pF at the voltages spanned by the action potential plateau and increases with progressive depolarization above 0 mV. Because membrane impedance is high during the plateau, I7 even a current of this relatively small magnitude might importantly influence the height and duration of the action potential.

Ionic Identity of the Background Current

The results shown in Figures 1 and 2 were obtained under ionic conditions which included Cl- in both the extracellular and pipette solutions. A time-independent Cl- current activated by cAMP has been identified recently in heart cells.10,11,35 Even though we did not purposely elevate cAMP in our experiments, we considered the possibility that the background current represents the basal level of Cl- current. The results in Figures 3 and 4 argue against this idea. Figure 3 shows the voltage pulse protocol and raw currents (panel A) and current-voltage relations from the same cell (panels B and C), all in the presence of nitrendipine. In panel B, addition of the Cl- channel blocker 9-AC (400 μM) did not appreciably affect the basal current-voltage relations, as can be seen by comparing control values (□) with those after the addition of 9-AC (×); nevertheless, Ba2+ was able to block not only ICl but also much of the current evoked at positive potentials (◇). Ba2+ is known not to affect cardiac cAMP-regulated Cl- channels.35 At a test potential of 40 mV, 9-AC was observed to reduce the whole-cell outward current by only 4.8±3.2 pA (mean±SD, n=4). To confirm that the concentration of 9-AC we used would have sufficed to inhibit Cl- current if it had been present, as expected from previous results,11 we purposely induced Cl- current by exposure to 1 μM isoproterenol in the presence of Ba2+. In panel C, comparison of the current-voltage curve in the control condition (×) with the curve obtained in the presence of isoproterenol (■) reveals the induction of a current that is active over a broad range of potentials and reverses near the Cl- equilibrium potential (ECl) (∼−4 mV); subsequent addition of 9-AC (◇) was then able to suppress the induced
current by 40–50% at all the voltages tested. Incomplete block of the isoproterenol-induced Cl− current has been observed previously and is at least partially attributable to the slow onset of block of the isoproterenol-induced current by 9-AC. The insensitivity of the basal background current to 9-AC and its blockade by Ba2+ are both inconsistent with the notion that it represents Cl− current. Although these results demonstrate that the currents in Figures 1 and 2 do not contain any significant contribution from the Cl− current, all subsequent experiments were performed in the strict absence of Cl− to minimize the possibility of anionic current contamination.

Figure 4 takes advantage of the observed block by low concentrations of Ba2+ to refine our characterization of the background current. Currents were measured before and after exposure to 1–2 mM Ba2+: the difference between the two sets of recordings is shown in Figure 4. Panel A bolsters the impression initially gleaned from Figures 1 and 2 that the currents are truly time independent on the cardiac time scale. Ba2+-sensitive current is prominent within 5 msec of the onset of depolarization and shows no clear tendency to turn on further or to inactivate with maintained depolarization. The average magnitude of the Ba2+-sensitive current density was 0.16±0.04 pA/pF at 0 mV and 0.52±0.21 pA/pF at +80 mV (n=13, mean±SEM), which are significantly greater than 0 pA (p<0.05 by t test). On repolarization to −40 mV, there is no decaying tail of outward current. This contrasts with the kinetics of activation and deactivation of the two components of the delayed rectifier, iK, and iKr, both of which display clear-cut tail currents and resolvable activation and deactivation kinetics. The time course of the outward current in Figure 4 fits much better with the properties of iKr channels, which are blocked by Ba2+ and exhibit kinetics and voltage dependence entirely consistent with those in Figure 4.22 The rapid kinetics of the instantaneous current in Figure 4 in whole-cell experiments are very similar to those reported previously in several cardiac preparations.19–21,24 To consolidate the identification of the macroscopic background current with iK, we specifically sought to exclude contributions from other known K+ currents, specifically iKATP, iK1, and IKs. The Na+-activated K+ channel13 was excluded since the [Na+]o was only 5 mM in our pipette solutions, a concentration five times lower than that required to induce this current.13

**Distinction of Background K+ Current From Other K+ Channels**

Although iKATP is not expected to be active under our experimental conditions,32 the sensitivity of the iKATP channels to ATP is reduced from the micromolar to the millimolar range after prolonged access into the intracellular environment.36 Therefore, we determined whether the background current is sensitive to glibenclamide, a blocker of ATP-sensitive K+ channels.37 Figure 5 shows that glibenclamide does not inhibit the currents evoked by depolarization, as shown by the representative step to 40 mV in panel A and by the complete current–voltage relation in panel B. The reduction in whole-cell outward current at 40 mV was insignificant (3.6±4.5 pA, mean±SD, n=3). In contrast to the insensitivity of the background current at positive potentials to glibenclamide, a modest inhibition of iK1 was observed at both −60 and −90 mV (panel A, left) at this high concentration of glibenclamide. These results, which were confirmed in two other cells, verify that the background current does not reflect iKATP.

We have already argued that the background current at positive potentials is not due to iK1, given the intense inward rectification of this current.29,38 The absence of outward current through iK1 channels is not only due to block of the permeation pathway by internal Mg2+ but also due to a dramatic decrease of open probability with depolarization.29,39,40 Nevertheless, it is conceivable that the background current at depolarized potentials may arise directly from iK1. To eliminate iK1, we took advantage of the fact that this current is inhibited by removal of external K+.41,42 Figure 6A shows representative membrane currents before (X) and after (□) K+ removal. Although the inward current evoked by hyperpolarization to −90 mV is markedly suppressed (left recording), as is the outward holding current at −40 mV, the current during depolarization to +40 mV is unaffected (right recording). The complete current–voltage relations before and after K+ removal (panel B) display precisely the behavior that would be expected from selective removal of iK1: the distinctive N shape is
abolished, leaving only a small inward current at negative potentials and a progressively increasing outward current at positive voltages.

The residual current after K\textsuperscript+ removal is not just a nonspecific leak: Figure 6C shows that the current–voltage relation at the more positive voltages in 0 K\textsuperscript+ (○) before the addition of Ba\textsuperscript{2+} is inhibited after the addition of 2 mM Ba\textsuperscript{2+}. The average magnitude of the current blocked by 2 mM Ba\textsuperscript{2+} in the absence of external K\textsuperscript+ was 106±59 pA at 40 mV and 145±54 pA at 70 mV (mean±SD, n=3); these values are significantly different from 0 (p<0.005 by regression analysis). Note that the current at test potentials below −30 mV was unaffected by the addition of Ba\textsuperscript{2+}. The persistence of the Ba\textsuperscript{2+}-sensitive current in 0 K\textsuperscript+ agrees with the observation that i_{Kp} is not suppressed by reduction of external K\textsuperscript+. Furthermore, the distinctive voltage dependence of the Ba\textsuperscript{2+}-sensitive current in 0 K\textsuperscript+ is entirely consistent with that predicted for the macroscopic correlate of i_{Kp}.\textsuperscript{22}

The final distinction that merits consideration is that between the background K\textsuperscript+ current and I\textsubscript{k}. Several features that have already been highlighted render I\textsubscript{k} unlikely to account for the background current. First of all, background current is active virtually instantaneously on depolarization, but I\textsubscript{k} activates slowly\textsuperscript{16,29}; second, there are no time-dependent changes on the time scale of our brief test pulses (<200 msec); third, no decaying outward tails are seen on repolarization to potentials positive to the K\textsuperscript+ equilibrium potential, E\textsubscript{k}, as would be expected for I\textsubscript{k}.\textsuperscript{16,29} Nevertheless, Figure 7 presents the most direct evidence against the idea that the background current is due to I\textsubscript{k}. This cell was studied in 0 K\textsuperscript+ to suppress I\textsubscript{k}. Background current was elicited by 180-msec depolarizing pulses (panels A and C), whereas much longer pulses (3 seconds) were used to evoke I\textsubscript{k} (panels B and D). Alternating between the two stimulation protocols, we contrasted the effects of 1 and 30 mM Ba\textsuperscript{2+} on the two types of currents. Panels A and C show that the background current was blocked by 1 mM Ba\textsuperscript{2+}, as observed previously (Figures 3, 4, and 6); no further inhibition was produced by Ba\textsuperscript{2+} concentrations as high as 30 mM. In contrast, panels B and D reveal that I\textsubscript{k} was only inhibited by approximately 30% by 1 mM Ba\textsuperscript{2+}. Even 30 mM Ba\textsuperscript{2+} did not suffice to block I\textsubscript{k} entirely. The insensitivity of I\textsubscript{k} to block by external Ba\textsuperscript{2+} was confirmed in a total of five cells. Because of the experimental design, the striking difference in Ba\textsuperscript{2+} sensitivity cannot be due to differences in test pulse voltage or ionic conditions or to intercellular variability.

**Contribution of Background K\textsuperscript+ Current to the Action Potential**

From our estimates of the size of the plateau current and its sensitivity to Ba\textsuperscript{2+}, the action potential duration is expected to be modulated by the addition of Ba\textsuperscript{2+} even when I\textsubscript{k} and L-type Ca\textsuperscript{2+} current have been blocked. In Figure 8A, an effect of the addition of Ba\textsuperscript{2+}...
on the action potential is clearly apparent in the presence of nitrendipine and with [K+]o of 5 mM. After the addition of 1 mM Ba2+, the action potential duration is prolonged by approximately 20%. The effect of the addition of Ba2+ begins very early in the repolarization process when the membrane potential is >0 mV. Under these conditions, neither I_{CI} nor I_K would be expected to contribute to the initial repolarization process. Nevertheless, to confirm that the effects in panel A are not due to block of I_{CI}, the effect of Ba2+ on the action potential was also examined in the absence of external K+. Panel B demonstrates that the action potential is prolonged, and the height of the plateau is elevated after the addition of 1 mM Ba2+, even when I_{CI} has been inhibited.

**Comparison of the Macroscopic Background Current and the Predicted Current Associated With i_{Kp}**

Do the rapidly activating Ba2+-sensitive plateau currents resemble the macroscopic currents expected from the single-channel properties of i_{Kp}? Figure 9 shows an example of the Ba2+-subtracted whole-cell background current (panel A) side by side with the ensemble-averaged current from a cell-attached patch containing an i_{Kp} channel (panel B). The remarkable similarity of the kinetic behavior, along with the Ba2+-sensitivity of i_{Kp}, makes a compelling case that the two currents are related or identical.

**Discussion**

The results presented above confirm the existence of a background plateau K+ current in guinea pig ventricles.

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**Figure 4.** Ba2+-sensitive plateau currents in the absence of Cl−. Panel A: Raw current tracings obtained by subtracting the currents measured after the addition of 2 mM Ba2+ from the currents measured before Ba2+ addition. Panel B: Graph showing the complete current density–voltage relation for the Ba2+-sensitive currents pooled from 13 cells. Nitrendipine (10 μM) was present in these experiments.

**Figure 5.** Test for current through ATP-sensitive K+ channels using 10 μM glibenclamide (GLIBEN) in the absence of Cl−. Panel A: Recordings showing the current response to a voltage step to 40 mV from a holding potential of −40 mV in the presence of 10 μM nitrendipine both before and after the application of 10 μM glibenclamide. Panel B: Graph showing the corresponding current–voltage relations over the full voltage range in the presence (×) and absence (control [CONT], □) of glibenclamide.
ular myocytes and demonstrate that this current is Ba-sensitive. The precise relation of the outward plateau current characterized in the present study to that previously described as the maintained component of $I_{\text{pump}}$ in other species, which is regulated and blocked independently from the time-dependent portion of $I_{\text{pump}}$, is unclear. By definition, the current characterized here is not $I_{\text{pump}}$ since its time course is not transient. There is, however, a convincing correspondence between the macroscopic background current that we have characterized and the previously described single-channel properties of $i_{\text{K,p}}$. Under experimental conditions in which the only Ba-sensitive current present is the background current (see Figure 6), the current–voltage relation is remarkably similar to that expected from the single-channel properties of $i_{\text{K,p}}$. The sensitivity to Ba of the plateau currents supports the contention that this current is carried primarily by K+, since many K+ channels are effectively blocked by Ba (References 47–49) as is $i_{\text{K,p}}$.

Although a number of other rapidly activating membrane currents have been previously described in cardiac myocytes, only $i_{\text{K,p}}$ appears to account for our results. The Na+-K+ pump, although capable of generating currents of the size observed in this study, is unlikely to be activated significantly under the ionic conditions used in our experiments; with only 5 mM Na+ in the pipette or in K+ conditions, pump currents are insignificant. Furthermore, the pump current is not Ba-sensitive. Similarly, the background current is unlikely to be carried by Na+-activated K+ channels, since the intracellular [Na+] must be at least a factor five times greater than that used in our intracellular solutions to evoke any significant current. In addition, currents across the membrane-glass seal resistance would be expected to reverse at 0 mV and would be at least an order of magnitude smaller than the Ba-sensitive currents we have measured at positive potentials (see “Materials and Methods”).

Although the intracellular [ATP], which is likely to be about the same as the concentration in our pipette solutions (2.5 mM), is well above the levels necessary to suppress activity of $I_{\text{KATP}}$, the possibility exists that the sensitivity of $I_{\text{KATP}}$ to ATP may decrease with time, as has been previously suggested. This was not the case in the present study, since 10 μM glibenclamide was without effect on the plateau currents. In general, the whole-cell recordings described in this study did not require experimental recording periods of more than
approximately 15 minutes; much longer times are needed to desuppress $I_{\text{K,ATP}}$.36

It is conceivable that the plateau currents reported here arise from current through $I_{\text{K1}}$ channels, but this is unlikely for two reasons. First, at the single-channel level, outward current through inward rectifier channels in the presence of intracellular Mg$^{2+}$ has never been convincingly demonstrated34 because of intense block of the outward currents by Mg$^{2+}$ as well as the rapid closing of the channel at potentials positive to $E_{\text{K}}$.34,38 Although Mg$^{2+}$ can influence both the permeation and gating properties of the inward rectifier channels, experimental and modeling data support the notion that little or no outward current passes through $I_{\text{K1}}$ channels at positive potentials under ionic conditions similar to those used in our experiments.38–40 The second reason to reject the notion that the plateau currents are related to inward rectifier channels is illustrated in Figure 6C. This figure confirms that a Ba$^{2+}$-sensitive current is still present at depolarized potentials in the absence of K$^+$, when the current through $I_{\text{K1}}$ is inhibited.41–43

Although the background current reported in this paper exhibits kinetic properties that are clearly distinct from those of the delayed rectifier, $I_{\text{K1}}$, its Ba$^{2+}$ sensitivity is also different from that of $I_{\text{K1}}$ as illustrated in Figure 7. The relative lack of sensitivity of the delayed

FIGURE 7. Effects of Ba$^{2+}$ on the instantaneous background current and the delayed rectifier current. Panel A: Recordings showing the reduction of outward background current from the control condition (●), after the addition of 1 mM Ba$^{2+}$ (▼) and 30 mM Ba$^{2+}$ (▲) for voltage steps to 40 mV from a holding potential of −40 mV. Panel B: Recordings showing the instantaneous background currents and the time-dependent delayed rectifier currents in the control condition (●) and in the presence of 1 mM Ba$^{2+}$ (▼) and 30 mM Ba$^{2+}$ (▲). Panel C: Graph showing the current–voltage relations, which were measured 30 msec after the start of the test pulse, with 0 mM (●), 1 mM (▼), and 30 mM (▲) Ba$^{2+}$. Panel D: Graph showing the current–voltage relation of the tail currents in the control condition (●), in 1 mM Ba$^{2+}$ (▼), and in 30 mM Ba$^{2+}$ (▲). Nitrendipine (10 μM) was present throughout.

FIGURE 8. Effect of Ba$^{2+}$ on action potentials in the presence and absence of external K$. Nitrendipine (10 μM) was present throughout. Panel A: Prolongation of the action potential duration, relative to the control condition (□), after the addition of 1 mM Ba$^{2+}$ (●) when 5 mM external K$^+$ was present. Panel B: Action potentials before (○) and after (●) the addition of 1 mM Ba$^{2+}$ in the absence of external potassium. Under these conditions, the action potential is again prolonged after the addition of Ba$^{2+}$. In the experiment shown in panel B, a hyperpolarizing current was introduced for a 400-msec period, just before the initiation of an action potential, to hyperpolarize the membrane to approximately −80 mV. This was necessary to remove inactivation of the Na$^+$ current and therefore render the cell excitible.
rectifier to Ba\(^{2+}\) has been reported previously in squid axons\(^{51}\) but, surprisingly, not in heart cells.

A large number of reports in the literature acknowledge the existence of an “instantaneously” activating outward current, although such a current has not been extensively characterized. In previous work on the delayed rectifier in cardiac muscle, this current can often be seen as a pedestal or offset underlying the time-dependent activation of \(I_{K}\).\(^{16,23,24,52}\) Delayed rectifier channels that activate very rapidly have been described previously in embryonic chick ventricular myocytes\(^{10}\); the rapid activation of these currents was thought to originate from the high probability of occupancy of closed states just before the open state at depolarized holding potentials (i.e., \(-50\) mV).\(^{10}\) However, an instantaneous component associated with the delayed rectifier seems unlikely in guinea pig myocytes, since neither component of \(I_{K}\), both of which have clearly measurable activation kinetics, is activated at our holding potential of \(-40\) mV.\(^{16,47}\) The instantaneous component that is often evident when recording delayed rectifier currents is clearly seen in Figure 7 to be Ba\(^{2+}\) sensitive, whereas \(I_{K}\) is not. This pedestal current is not the instantaneous current reported to arise from changes in the gating properties of the delayed rectifier from squid axons when the external \([Ca^{2+}]\) is reduced to zero,\(^{55}\) since all the experiments shown here were performed in the presence of 2 mM Ca\(^{2+}\). Interestingly, a large pedestal current has also been seen to develop at high intracellular \([Ca^{2+}]\).\(^{52}\) Although \(i_{K,p}\) does not require elevated cytoplasmic \([Ca^{2+}]\) for activity,\(^{22}\) a modulatory role of intracellular Ca\(^{2+}\) has not been excluded.

The current–voltage relation observed for the Ba\(^{2+}\)-sensitive plateau current is markedly different from that expected from the single-channel recordings of \(i_{K,p}\), which are also Ba\(^{2+}\) sensitive. An equally noteworthy correspondence between the plateau currents and \(i_{K,p}\) is the similarity of the kinetics of the macroscopic and ensemble-averaged currents, as demonstrated in Figure 9. Is there quantitative consistency between the observed macroscopic plateau currents and predictions based on the single-channel recordings of \(i_{K,p}\)? On the basis of the resistance of the pipettes used by Yue and Marban\(^{22}\) (i.e., 1–4 M\(\Omega\)) and their stated probability of finding \(i_{K,p}\) channels in cell-attached patches (approximately 0.1), we estimate that the number of channels per cell is roughly 40–120. This estimate assumes that 1) the total membrane area of our cells was approximately 1.2\(\times\)10\(^6\) \(\mu\)m\(^2\) (assuming 120 pF per average cell and a specific capacity of 1 \(\mu\)F/cm\(^2\)), 2) half of the membrane surface area of a myocyte is T tubular\(^{54}\) (i.e., inaccessible to cell-attached recording), and 3) pipettes of this size seal with sarcolemmal membrane areas of 5–15 \(\mu\)m\(^2\).\(^{55}\) Given a unitary current of 1.6 pA and an average open probability of 0.48 for \(i_{K,p}\) at +30 mV,\(^{22}\) we estimate a current density of 0.25–0.76 pA/pF; this is reassuringly consistent with the measured macroscopic plateau current density of 0.24±0.05 pA/pF at this voltage.

In considering the likely physiological role of \(i_{K,p}\), allowance must be made for the fact that the present experiments were conducted at room temperature. Because the delayed rectifier turns on very slowly at room temperature, \(I_{K,p}\) and \(I_{K}\) were clearly separated kinetically (Figure 7). At physiological temperatures, \(I_{K,p}\) remains present, but \(I_{K}\) activates more rapidly and increases in magnitude (M. Sanguinetti, University of Utah, Salt Lake City, personal communication), favoring its ability to influence repolarization. Likewise, the action potential results should be interpreted cautiously, given the highly artificial conditions (i.e., 10 \(\mu\)M nitrendipine and 0 K\(^{+}\)) used to check the effect of selective \(i_{K,p}\) blockade.

Understanding of the relative contributions of the various K\(^{+}\) channels to repolarization will be dramatically improved as selective pharmacological or genetic (e.g., antisense messenger RNA) blockers become available. In this context, it is notable that the recently cloned guinea pig homologue of K\(_{V1.5}\), when expressed in oocytes, exhibits time and voltage dependence values virtually identical to those of \(i_{K,p}\).\(^{27}\)

The magnitude of the plateau current was found on average \((n=13)\) to be approximately 0.24 pA/pF at a membrane potential of +30 mV. Although this amount of current can significantly influence action potential duration, not all cells showed a sizable background K\(^{+}\) current. Consequently, in those cells in which the current was most clearly observed, it was at least twice as large as one would expect from the average results in Figure 4. This may be related to known regional differences within the heart. The rates of repolarization are known from electrocardiography to proceed from the apex to the base of the heart, and it is this phenomenon that is responsible for the generation of the upright “T
wave". From this and other more direct lines of evidence we may infer that regional differences in channel composition and regulation exist in the heart. This may explain, in part, the variability in the amplitude of the background current, but we have no direct evidence to support this possibility. Finally, it should be emphasized that the estimated membrane conductance associated with the background macroscopic plateau currents is approximately 300 pS at a membrane potential of +30 mV. As a consequence, the membrane resistance during the plateau of the action potential is expected to remain very high (i.e., approximately 3 GΩ) in spite of the presence of these plateau currents.

In conclusion, we have characterized the background K+ current that is present in guinea pig myocytes. Because it appears to be the whole-cell correlate of the single-channel $I_{Kp}$, we propose the nomenclature $I_{Kp}$ for this current. $I_{Kp}$ can significantly alter action potential duration, as shown in Figure 8, and therefore merits further study with regard to its possible physiological role in the regulation of action potential duration. Finally, $I_{Kp}$ should be recognized as a possible target of the enlarging family of therapeutic agents designed to prevent ventricular arrhythmias by prolonging action potential depolarization (class III antiarrhythmics).

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