brief communication

# Dynamics of the inward rectifier $K^+$ current during the action potential of guinea pig ventricular myocytes

Jose Ibarra, Gregory E. Morley, and Mario Delmar
Department of Pharmacology, SUNY Health Science Center. Syracuse, New York 13210 USA

ABSTRACT The potassium selective, inward rectifier current ($I_{K1}$) is known to be responsible for maintaining the resting membrane potential of quiescent ventricular myocytes. However, the contribution of this current to the different phases of the cardiac action potential has not been adequately established. In the present study, we have used the action potential clamp (APC) technique to characterize the dynamic changes of a cesium-sensitive (i.e., $I_{K1}$) current which occur during the action potential. Our results show that (*a*) $I_{K1}$ is present during depolarization, as well as in the final phase of repolarization of the cardiac action potential. (*b*) The current reaches the zone of inward-going rectification before the regenerative action potential ensues. (c) The maximal outward current amplitude during repolarization is significantly lower than during depolarization, which supports the hypothesis that in adult guinea pig ventricular myocytes, $I_{K1}$ rectification is accentuated during the action potential plateau. Our results stress the importance of $I_{K1}$ in the modulation of cell excitability in the ventricular myocyte.

## INTRODUCTION

It is widely accepted that the potassium selective, inward rectifier current ($I_{K1}$) is the major determinant of the resting potential in the mammalian ventricular myocyte (Pennefather and Cohen, 1990). Results obtained in this and other laboratories suggest that $I_{K1}$ also plays an active role during action potential depolarization (Tourneur, 1986; Delmar et al., 1989*a*), and contributes to the final phase of action potential repolarization (Tourneur et al., 1987). Such results were obtained using conventional current and voltage clamp techniques, and the inferences derived from them await confirmation in a system in which a more direct analysis of current dynamics may be performed.

Recent evidence obtained in chick embryo cardiac cells (Mazzanti and DeFelice, 1990) also suggests that, although the inward rectifier channels are essentially time-independent in the voltage region of the action potential (Tourneur et al., 1987), the inward-going rectification of the channel can be accentuated by the cellular events which occur during the action potential plateau. The latter observation has not been tested for the whole-cell $I_{K1}$ of adult, mammalian myocytes maintained at physiological concentrations of extracellular potassium.

The action potential clamp (APC) techniques (Fischmeister et al., 1984; deHaas and Vogel, 1989) offer a valuable approach to the study of the dynamics of specific currents as they actually occur during the action potential. This paper describes the use of the whole cell APC technique (Starzak and Starzak, 1976, 1978; Cole, 1980; deHaas and Vogel, 1989; Doerr et al., 1989; Doerr et al., 1990) to analyze the behavior of $I_{K1}$ during the cardiac cycle of adult guinea pig ventricular myocytes. Our results reveal the specific contribution of $I_{K1}$ to the different phases of the cardiac action potential. The data also show that the maximal outward $I_{K1}$ during repolarization is significantly less than during subthreshold depolarization, just before activation of the cell.

Address correspondence to Dr. Delmar.

## METHODS

### Cell dissociation and recording procedures

Experiments were carried out in freshly-dissociated guinea pig ventricular myocytes. Details of the dissociation procedure are given in a previous publication from our laboratory (Delmar et al., 1989*a*). The composition of the Tyrode solution was (in mM): NaCl, 150; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 1.0; $NaHCO_3$, 5.8; $NaH_2PO_4$, 0.4; Glucose, 5.5; HEPES, 5.0; pH was balanced to 7.4. Temperature was maintained at 35°C.

Recordings were obtained using sylgard-coated suction pipettes filled with an internal pipette solution containing (in mM): KCl, 150; $MgCl_2$, 1.0; EGTA, 5; HEPES, 5; β-OH-butyric acid, 2.0; ATP (disodium salt), 5.0; phosphocreatine (disodium salt), 5.0. The pH was balanced to 7.2. Electrodes were coupled to an Axoclamp 2A amplifier operating in the current clamp or voltage clamp mode.

### Action potential clamp

The whole-cell APC technique consists of voltage clamping the cell membrane to its own action potential (deHaas and Vogel, 1989; Doerr et al., 1990). Membrane potential recordings were obtained from cardiac myocytes paced repetitively at a constant cycle length of


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700–1,000 ms with current pulses (duration, 40 ms) of just-threshold amplitude, applied through a patch pipette in the current clamp configuration. Long pulse durations were used to induce prolonged foot potentials, similar to those recorded from well-polarized cells during impulse propagation across a heterogeneous conduction pathway (Jalife, 1983; Delmar and Jalife, 1990; Hill et al., 1990; Davidenko et al., 1991 [in press]). A computer (Zenith Z248) was used to store (via a 12 bit analog/digital converter; DAS-20; Metrabyte; sampling rate 10 KHz) several action potentials. The amplifier was then switched to single electrode voltage clamp mode and the recorded trace was played back (via a 12-bit digital/analog converter) to act as the voltage command for the preparation.

We have often observed that the resting potential of the myocyte shifts slightly (within 2 mV) during control recordings, particularly when the cell is paced repetitively at a relatively short ($\leq 1$ s) cycle length (Lorente et al., 1991). Thus, for the control runs, we always adjusted the holding potential at the onset of recording to match (within 1 mV) the value of resting potential that was measured immediately before switching to voltage clamp mode. Consequently, holding current during control conditions was equal to zero.

Cesium chloride (15 mM) was used as an $I_{K1}$ blocker (Tourneur et al., 1987; Oliva et al., 1990; see Discussion). Active currents were stored on a videotape-based system (Neurocorder Model DR 484) and later analyzed with the computer using the CLAMPEX and CLAMPFIT routines of the pCLAMP system. Dynamic $I_{K1}$ current-voltage (*IV*) relations were constructed by correlating the value of membrane potential wth the value of $I_{K1}$ at each sample point.

A variable gain amplifier was included in the action potential clamp circuit to ensure that the voltage signal delivered to the cell as command potential had the same amplitude (within 1 mV at the action potential plateau) as the one generated by the cell during current clamp recording (Doerr et al., 1990). In our initial series of experiments ($n = 11$), the variable gain amplifier was interposed between the output of the D/A converter and the external command input of the patch clamp amplifier operating in the voltage clamp mode. In these experiments, 10 sequential recordings were averaged to obtain the pertinent current and voltage data to generate the $I_{K1}$ *IV* relations. In a second series of experiments ($n = 9$), the variable gain amplifier was interposed between the output of the patch clamp amplifier operating in current clamp mode and the input of the A/D converter (during action potential acquisition). This modification allowed us to take advantage of most of the voltage range of the A/D board, thus enhancing the resolution of the voltage signal and reducing the amplitude of the individual voltage steps. This procedure led to a drastic reduction in the noise amplitude of the current traces during action potential clamp. Noise was further reduced by placing a Bessel filter (1–2 KHz cut-off frequency) between the output of the D/A converter and the external command input of the voltage clamp amplifier. Given the reduction in noise amplitude, dynamic $I_{K1}$ *IV* relations could be generated from single traces. Similar results were obtained regardless of whether averaged or single traces were analyzed.

## RESULTS

### Dynamics of $I_{K1}$ in the cardiac cycle

An example of our results is presented in Fig. 1. In *A*, the trace labelled AP illustrates an action potential acquired from a guinea pig ventricular myocyte using the whole-cell current clamp method. After switching to the voltage clamp mode, the same action potential was used as the voltage command for the cell membrane, eliciting the current labelled $I_p$. This current trace mimics the original stimulating pulse, except for a small offset from the baseline observed during the action potential plateau (see Discussion). Trace $I_{Cs}$ was recorded 3 min after addition of 15 mM cesium to the superfusate. In this case, the voltage clamp amplifier was forced to compensate for both the absent pulse and the Cs-sensitive (i.e., $I_{K1}$) currents. By digitally subtracting trace $I_p$ from $I_{Cs}$ we obtained a trace of $I_{K1}$ throughout the different phases of the cardiac cycle (trace labelled $I_{K1}$). Note that, in the APC technique, the amplifier compensates for absent currents, and thus, the conventional polarity of the current is inverted (deHass and Vogel, 1989; Doerr et al., 1990).

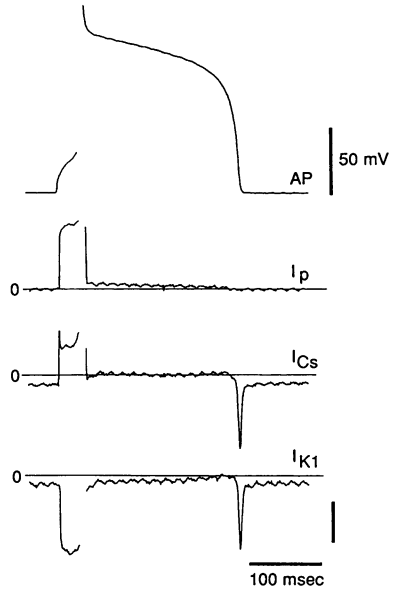


FIGURE 1 Action potential clamp recordings of $I_{K1}$. Top trace depicts the action potential (AP) used as voltage command for the same cell. $I_p$ and $I_{Cs}$ represent, respectively, single-current traces obtained under APC before and after addition of 15 mM cesium to the superfusate. $I_{K1}$ represents the Cs-sensitive current. Bottom vertical calibration: 500 pA for trace $I_p$ and 300 pA for traces $I_{Cs}$ and $I_{K1}$. Membrane outward currents are represented as downward deflections. The horizontal lines across the $I_p$, $I_{Cs}$ and $I_{K1}$ traces indicate zero current level.

A plot of voltage (AP) versus current ($I_{K1}$) at each point in time yielded the dynamic $I_{K1}$ *IV* relation (Mazzanti and DeFelice, 1990), shown in Fig. 2. In fact, this technique allowed us to obtain an *IV* relation for $I_{K1}$ both during depolarization (*open squares*) and during repolarization (*crosses*). Data obtained during the action potential upstroke (between −54 mV and 50 mV) were affected by the lack of voltage control during the rapid depolarization (Doerr et al., 1990) and thus were not included in the plot (see Discussion).

In Figs. 1 and 2, the holding current for the $I_{Cs}$ and $I_{K1}$ traces was slightly outward during diastole. This has been previously shown for APC experiments in which all (or most) outward conductances were blocked (see Fig. 2 *D* of Doerr et al., 1990) and it probably reflects the fact that the resting membrane potential is not identical to the equilibrium potential for $I_{K1}$. As shown in Fig. 2, at the subthreshold level of potentials, the inward rectifier increased progressively during the pulse and reached the zone of inward-going rectifcation before the fast action potential upstroke ensued. Current was almost negligible during the action potential plateau but increased again as the cell repolarized beyond −40 mV; in contrast with what would be expected from a time-independent current, the *IV* relation during depolarization was clearly distinct from that during repolarization. The following characteristics were consistently observed in our experiments ($n = 20$): (*a*) the IV curves intersected 10–15 mV above the resting potential

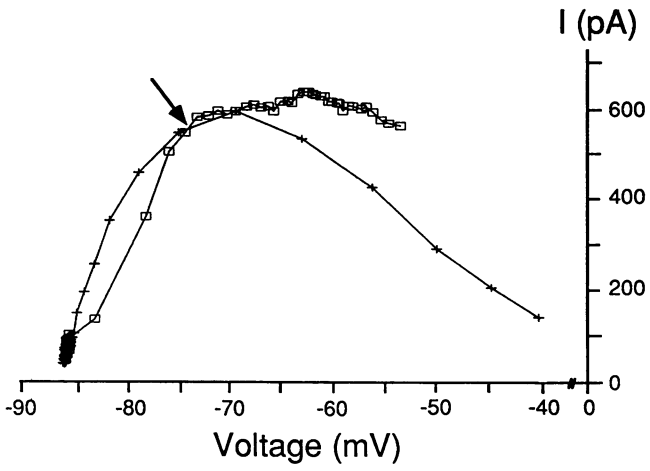


FIGURE 2 Dynamic $I_{K1}$ IV relation. Data points were obtained during either subthreshold depolarization (*open squares*) or during phase-3 repolarization (*crosses*) of the action potential shown in Fig. 1. Following the conventional representation, outward currents are considered to be of positive sign.

(indicated by an *arrow* in Fig. 2). (*b*) For voltage values between the intersecting point and activation threshold (−54 mV in the case shown in Figs. 1 and 2), the outward current obtained during depolarization was larger than that measured following the action potential plateau. (*c*) Repolarization current was larger than depolarization current between resting potential and the intersecting point. (*d*) The voltage value corresponding to the maximal outward current during repolarization was more negative than the one obtained during depolarization. (*e*) The maximal amplitude of outward current reached during subthreshold depolarization (703 ± 306.8 pA. X ± SD; $n$ = 20) was larger than that measured during repolarization (514 ± 247 pA). The difference between the two groups was highly significant (two tailed $p$ value = 0.0001) as determined by a paired $t$ test. The relative difference varied widely from one experiment to another, the average being 27.9 ± 11.6%. These differences strongly suggest that events occurring during the action potential plateau significantly alter the conductance of the $I_{K1}$ channel (see also Mazzanti and DeFelice, 1990).

## DISCUSSION

The action potential clamp technique offers a valuable approach to the study of the dynamics of specific currents as they actually occur during the action potential. This can be carried out by either allowing the cell to clamp to its own action potential while recording the membrane channel activity with a cell-attached pipette (i.e., action potential patch clamp, or patch-APC; Fischmeister et al., 1984) or by digitizing the action potential and then using that action potential as the voltage command for the same cell (whole cell-APC; Starzak and Starzak, 1976, 1978; Cole, 1980; deHaas and Vogel, 1989; Doerr et al., 1989, 1990).

Whole-cell APC recordings are useful for the study of currents that are difficult to identify at the single-channel level. For example, in the case of $I_{K1}$, single-channel outward currents are impossible to detect at a normal extracellular concentration of potassium ($[K]_0$), making it necessary to raise $[K]_0$ to unphysiological levels (11.3 mM or above; Mazzanti and DeFelice, 1988, 1990). This procedure not only alters channel conductance (Sakmann and Trube, 1984; Matsuda, 1991) but it also shifts the reversal potential of the current and, consequently, alters the voltage gradient at which the channel operates. The latter may be important since the kinetic properties of the channel are known to be voltage dependent (Ishihara et al., 1989; Oliva et al., 1990). In addition, the shift of reversal potential due to high $[K]_0$ would prevent the study of the dynamics of outward $I_{K1}$ in the voltage range of the subthreshold response. In summary, patch-APC and whole-cell APC techniques complement each other, and they both represent excellent tools in the study of the dynamic properties of membrane ionic currents.

### Limitations of the action potential clamp technique

A common problem that we have faced in our experiments is the loss of voltage clamp control during the action potential upstroke, probably due to the high frequency and large amplitude of the command signal during that phase of the action potential (Doerr et al., 1990). To avoid errors of interpretation, we systematically discarded currents obtained during the action potential upstroke. Consequently, we could not determine the exact shape of the negative slope region during depolarization.

A small current (which we shall refer to as "offset current") was often observed during the action potential plateau in normal Tyrode solution (see $I_p$ trace, Fig. 1). A small offset from the baseline was also found by deHaas and Vogel (1989) in their APC experiments of single nerve axon. In some experiments we repeated the APC protocol after Cs washout. In those instances, the offset current was very similar to the one initially found for the $I_p$ trace.

The presence of an offset current could be explained by at least two mechanisms. First, it is possible that this current results from temporal variations in the background conductance (Belles et al., 1987). However, it is unlikely that an increase in background conductance would occur in our experimental conditions (pipette [ATP] = 5 mM) in the brief time (<3 min) elapsed between current clamp recording and the moment of acquisition of $I_p$. A second possibility is that the offset current results from a small (<1 mV) mismatch between the original action potential (as recorded from the

cell) and the command signal delivered to the cell during APC. We routinely adjusted for possible differences (see Methods). However, small unresolved mismatches may have remained.

Regardless of its underlying mechanism, the characteristics of the offset current were such that it would not be expected to significantly alter the shape of the $I_{K1}$ *IV* relation. Indeed, the offset current was still present after Cs washout and is therefore possible that it was also present during Cs superfusion; in that case, the offset current would be cancelled out during the subtraction procedure. Moreover, even if the offset current was not completely eliminated by the subtraction, it would only alter the amplitude of the subtracted currents recorded in the plateau range of potentials. Yet, in this paper, we only analyze the time and voltage dependence of $I_{K1}$ for voltages more negative than −40 mV. Finally, we should point out that in two experiments, no offset current was detected. Results from the latter experiments were similar to those illustrated in Figs. 1 and 2.

The above limitations notwithstanding, the data presented in this paper do show the dynamic contribution of a Cs-sensitive current to the different phases of the cardiac action potential. As discussed below, the evidence available in the literature strongly supports the hypothesis that such a Cs-sensitive current is largely carried through inward rectifier, $I_{K1}$ channels.

## Cesium selectivity

The applicability of the APC technique to the study of individual ionic currents is highly dependent on the specificity of the channel blocker being used. An excellent discussion on the nature of Cs-sensitive currents can be found in Oliva et al. (1990). These authors provide strong arguments in support of the premise that, for voltages more negative than −20 mV, the cesium-sensitive current is largely (if not exclusively) $I_{K1}$. A similar conclusion was drawn by Tourneur et al. (1987) when studying guinea pig ventricular myocytes.

In the studies cited above, Cs-sensitive currents were recorded in the presence of calcium channel blockers. In our case, calcium currents were still functional. However, it is unlikely that calcium currents were affected by cesium because no sizable inward currents were detected at any time in the subtracted trace. In addition, Ca-channel permeability studies have shown that cesium is the least permeable of all cations (Hess et al., 1986). Moreover, we are not aware of any studies indicating a blocking effect of cesium on calcium currents. In fact, cesium-containing solutions are commonly used to study the functional properties of calcium currents in cardiac cells (see e.g., Tseng, 1988; Hartzell and White, 1989).

The characteristics of the $I_{K1}$ *IV* curves obtained by digital subtraction of Cs-sensitive currents (see, for example, Tourneur et al., 1987; Oliva et al., 1990) are quite similar to those observed by measuring the difference between records obtained at 14 mM $[K]_0$ and those obtained in K-free solution, using the oil-gap technique (see, e.g., Ishihara et al., 1989).

In summary, our results as well as those of others strongly suggest that most (if not all) of the Cs-sensitive outward current that was recorded in our experiments between −40 mV and resting potential indeed corresponds to the whole-cell $I_{K1}$ previously identified through more conventional voltage clamp techniques (Pennefather and Cohen, 1990).

## Role of $I_{K1}$ in cell depolarization

Our results demonstrate that, when just-threshold current pulses are used to activate the cell, the membrane potential enters the negative slope region of the $I_{K1}$ *IV* relation before the rapid upstroke ensues (see Fig. 2). These data conclusively demonstrate the active role of $I_{K1}$ in determining the amplitude and shape of the subthreshold depolarization (Delmar et al., 1989*a*), and strongly support the hypothesis that the negative slope region of $I_{K1}$ contributes to the overall depolarization of the cell (Tourneur, 1986).

Cell excitability has generally been associated with the ability of inward currents to generate an action potential upstroke. Yet, even in the presence of normal sodium channels, the cell will not generate an action potential if it fails to depolarize from its resting level to threshold. It is in this regard that input resistance may play a role in the excitability of cardiac cells.

Under normal circumstances, the propagating current is much larger than the minimum current required for excitation and, consequently, the characteristics of $I_{K1}$ would be irrelevant to the overall process of activation. However, when propagation is impaired, electrotonic depolarizations of long duration can precede the development of the active response (see Jalife, 1983; Delmar and Jalife, 1990, for review). In those instances, the dynamic properties of $I_{K1}$ play a critical role in determining the amplitude and shape of the subthreshold response (Tourneur, 1986; Delmar et al., 1989*a*, *b*; Lorente et al., [1991]) and consequently, determine the success or failure of activation.

## $I_{K1}$ conductance is reduced by events occurring during the action potential plateau

We consistently observed *IV* relations which showed a more pronounced inward-going rectification during action potential repolarization. This observation corre-

lates well with that of Mazzanti and DeFelice (1990), showing that $I_{K1}$ rectification is accentuated following the action potential plateau.

We may speculate that the increased rectification observed after active depolarization occurs as a consequence of the voltage-dependent blocking effect of cytosolic free calcium ions on the conductance of the $I_{K1}$ channels (Mazzanti and DiFrancesco, 1989). An argument against this possibility is the fact that our experiments were carried out in the presence of 5 mM EGTA in the pipette solution, which would presumably chelate $Ca_i$. Yet, we can not be certain as to whether the EGTA was able to diffuse into the intracellular space and abolish the systolic Ca transients. Further experiments in which the cell is actively dialyzed with a known concentration of $Ca^{2+}$ would be necessary to test this hypothesis. Moreover, it is possible that, as shown by Mazzanti and DeFelice (1990) for chick embryo myocytes, $I_{K1}$ channel block during the action potential plateau is caused by the same calcium ions entering the cell during the action potential; in that case, it is possible that calcium ions could escape EGTA chelation because they are confined to the restricted subsarcolemal space.

Another possibility is that the reduced $I_{K1}$ is a consequence of the time- and voltage dependence of Mg-mediated open channel block (Matsuda, 1988) and/or a result of the voltage- and time-dependent gating properties of the channel (Kurachi, 1985). Indeed, the combination of particle block and gate closing may lead to further reduction in open channel probability as the membrane depolarizes into the voltage levels of the action potential plateau (Ishihara et al., 1989), with a consequent reduction in whole-cell outward current during repolarization (Ishihara et al., 1989; Oliva et al., 1990). Yet, partial blockade of open channels may explain the paradoxical increase in outward current during repolarization at the most negative levels of membrane potential (Oliva et al., 1990). Further implementation of kinetic models in which both particle block and channel gating are taken into account (such as those of Ishihara et al., 1989, or Oliva et al., 1990) will be necessary to advance our understanding of the dynamic behavior of $I_{K1}$ during the cardiac action potential.

## CONCLUSIONS

Our results show that $I_{K1}$ dynamically contributes to the electrophysiological events of the cardiac cycle. Indeed, $I_{K1}$ participates in the regenerative depolarization process and, therefore, may play a role in cell excitability. It also provides outward current during the final phase of action potential repolarization. Finally, our results show that, in addition to its well-known voltage dependence, $I_{K1}$ outward conductance is also a function of time. The implications that this temporal component may have on the origin and maintenance of normal and abnormal cardiac rhythms, remains to be determined.

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