Two Types of Delayed Rectifying K⁺ Channels in Atrial Cells of Guinea Pig Heart

Minoru Horie, Seiji Hayashi, and Chuichi Kawai

Third Department of Internal Medicine, Faculty of Medicine, Kyoto University, Kyoto, 606 Japan

Abstract Whole-cell clamp experiments revealed the double exponential deactivation of the delayed rectifier K⁺ current in single guinea pig atrial myocytes. Two types of K⁺-selective channels were identified by applying repolarizing voltage pulses to cell-free patches from atrial cell membrane, where inward-rectifier, ATP-sensitive, and muscarinic K⁺ channels were all inactivated. Under a symmetrical 150 mM K⁺ condition, single channel conductances of the channels were 10 and 3 pS. The reversal potential obtained from the unitary current-voltage relation coincided with the equilibrium potential for K⁺. Ensemble averages of both types of single channel currents showed deactivation kinetics upon hyperpolarizing to potentials between −40 and −120 mV. The more positive the pre-pulse potential, the greater was the peak ensemble current. It is concluded that these two channels are responsible for atrial delayed rectifying K⁺ currents.

Key words: guinea pig atrial myocyte, patch-clamp techniques, delayed rectifier K⁺ channels, single-channel currents, activation gate.

In the myocardium several types of delayed rectifying outward currents are known to contribute to the repolarization phase of action potentials (Noble, 1984). Voltage clamp studies have demonstrated that tails of delayed rectifying currents consist of two (fast and slow) components in sheep cardiac Purkinje fibers (Noble and Tsien, 1969), frog atrial myocardium (Ojeda and Rougier, 1974; Brown et al., 1977; Goto et al., 1983), rabbit sino-atrial node (Nomura and Irisawa, 1976; DiFrancesco et al., 1979) and feline ventricular muscle (McDonald and Trautwein, 1978). These studies at the macroscopic current level suggest that delayed rectifying currents are carried through two (or more) types of channels operating in parallel with different kinetic parameters. In the present study, using patch-clamp techniques, we present direct evidence for the coexistence of two types of K⁺ channels responsible for delayed rectifying currents in guinea pig atrial cells. A preliminary report has appeared in abstract form (Horie, 1989).

Received for publication May 12, 1989

479
MATERIALS AND METHODS

Single atrial cells were isolated from adult guinea pig hearts using Langendorff's perfusion technique with collagenase (Yakult, Japan) as previously described (Horie and Irisawa, 1989). Membrane currents were recorded in the whole-cell, cell-attached and inside-out configurations (Hamill et al., 1981) by means of a patch clamp amplifier (List Electronics, EPC-7).

In whole-cell experiments, cells were superfused with a warm Tyrode solution (33°C). Its composition was (in mM): 136.5 NaCl, 0.3 NaH₂PO₄, 5.4 KCl, 1.8 CaCl₂, 0.5 MgCl₂, and 5 N-2-hydroxyethyl piperazine-N'-2-ethanesulfonic acid (HEPES)/NaOH (pH 7.4). Pipettes had relatively large tips (resistance 2–3 MΩ) and the content of the pipette solution was (in mM): 110 K-aspartate, 20 KCl, 3 MgCl₂, 3 K₂ATP, 5 EGTA, 5 Na₂-creatine phosphate, 0.2 Na₂GTP, and 5 HEPES/KOH (pH 7.4).

Single channel currents were recorded from both cell-attached and inside-out patches. Patch pipettes were filled with a solution containing (in mM): 150 KCl, 1.8 CaCl₂, 5 HEPES/KOH (pH 7.4). The shank of pipettes was coated with silgard. To abolish the transmembrane potential, cells were superfused with a high K⁺ bath solution (22–24°C), the composition of which was (in mM): 150 KCl, 1 ethylene glycol-bis(β-aminoethyl ether) N,N'-tetraacetic acid (EGTA), 5 HEPES/KOH (pH 7.4). During the recording from cell-free patches, 2 mM K₂ATP (Sigma) was added to the bath solution to suppress the activation of ATP-sensitive K⁺ channels (Nomura, 1983). The holding potential ($V_h$) was varied from −60 mV to +40 mV, and hyperpolarizing test pulses ($V_t$) of 300–500 ms duration were applied to the patch pipette at intervals of 2–10 s.

Current data was stored on a video tape via a Pulse Code Modulation system (Sony PCM501), and later was analyzed by a computer (NEC PC-98XL) at the sampling rate of 0.5 or 1 kHz through a Bessel type filler (NF FV-624). The unit amplitude of the open-channel currents was determined as a mean of the difference between the baseline (no channel open) and the open state where one channel was opened. The half amplitude threshold method (Colquhoun and Sigworth, 1983) was used as a detection criterion for channel openings in the calculation of the open and closed time histograms. Numeral data were given as mean ± standard deviations (n: the number of observations).

RESULTS

Two components in whole-cell current

Since two components of delayed rectifier tail currents reported in various cardiac preparations have not yet been shown in single guinea pig atrial cells, we first conducted whole-cell clamp experiments. Figure 1A depicts a representative result, where delayed rectifying outward currents were elicited by depolarizing the membrane from the holding potential of −30 mV to various potential levels for
Fig. 1. Whole-cell tail current analysis. A: typical whole-cell current traces in a single atrial cell, elicited by the pulse protocol as shown in the upper panel. Arrowhead and dotted line indicate the zero-current level. B: semilogarithmic plots of outward tail current repolarized from +100 mV to −30 mV in the panel A. The current trace is digitized and illustrated by dots. Two straight lines were drawn using a least-square method, thereby giving double exponential function for the tail current.

2s. As illustrated in Fig. 1B, an outward tail current upon repolarization from +100 mV to −30 mV could approximately be expressed by a double exponential function. Time constants for the fast phase (τf) and for the slow phase (τs) were 128 and 470 ms in this case, respectively. In 6 cells, τf and τs averaged 95.8 ± 11.3 and 462.8 ± 51.1 ms. Thus, in guinea pig atrial cells, delayed rectifier tail currents consist of two components.

Based on the above whole-cell clamp findings, we next tried to record single-channel currents under the symmetrical 150 mM K+ conditions, where the activity through the delayed rectifier channel would decay upon repolarization from +10 mV to −80 or −100 mV. Figure 2 demonstrates original current traces obtained in the inside-out mode (A, C) and their ensemble average currents (B, D). Two types of channel openings were observed: one occurred in bursts with brief closings (e.g., see the 5th trace in panel A) and another with a smaller unit amplitude and longer life time (e.g., see the second trace in panel A, where no larger conductance channels are open).

Lager conductance channel

We first focused on the channel with a larger conductance. The events were clustering at the beginning of each trace but less often observed near the end of
Fig. 2. Two types of channels open upon hyperpolarization in atrial cell membrane. Original single-channel current traces (A, C) and ensemble average currents (B, D) recorded from an inside-out patch at $V_i$ of $-80$ (A, B) and $-100\,\text{mV}$ (C, D). Ensemble average currents were calculated from 143 and 85 current frames. Arrows and solid lines indicate the zero current level. Dotted lines with arrowheads indicate open-channel currents with larger conductances and dotted lines those with smaller conductances. Current transient artifacts, which resulted from the abrupt change of potentials, were subtracted from original traces. Note simultaneous openings of both channels (e.g., the second trace in C). Time and current calibrations are given to the bottom of each column. Vertical bars indicate $1$ (A, C) and $0.1\,\text{pA}$ (B, D). Unitary current-voltage relationships of large conductance channel (E) at external $K^+$ concentrations of $150\,\text{mM}$ (filled squares) and of $300\,\text{mM}$ (open squares).

test pulses (e.g., see the 5th, 7th, and 8th traces in panel A, where no smaller conductance channels are open). The number of channels present was at most two in most experiments. Unit amplitudes became greater at more negative $V_i$. Filled squares in Fig. 2E depict the unitary current-voltage ($I$-$V$) relationship determined from original current traces which contained openings of the larger conductance channels alone. The single channel conductance was $10\,\text{pS}$. The mean value was $10 \pm 1\,\text{pS}$ ($n=12$). Single channel currents at potentials more positive than $+40\,\text{mV}$ were so small and variable in the amplitude that identification of unitary currents was impossible. Therefore, the reversal potential was obtained by extrapolating the $I$-$V$ relationship at negative potentials to the zero current axis. The reversal potential was $0\,\text{mV}$, which is identical to the $K^+$ equilibrium potential ($E_K$).

Open squares in Fig. 2E show a representative $I$-$V$ relationship at the extracellular $K^+$ concentration ([K+]$_o$) of $300\,\text{mM}$. Mean single channel
Fig. 3. Single channel kinetics in 10 pS-channel. Open and closed time histograms calculated from current data at $V_r = -100$ mV. The open time distribution was fitted by a single exponential function with a time constant of 9 ms ($\tau_o$, A) using a bin width of 2 ms. The closed time distribution was fitted using a bin width of 4 ms, giving a time constant for slow component of 37 ms ($\tau_{c1}$, B). Extrapolation of the exponential curve to time zero revealed the remaining excess events with shorter closed times. Subtracting the slow component from the histogram yielded the fast component with a time constant of 1.2 ms ($\tau_{cf}$, C) using a bin width of 0.4 ms.

conductance increased to $17 \pm 1$ pS ($n = 5$), and the reversal potential shifted to $+22$ mV, which is close to $E_K (=17$ mV). These results indicate that the 10 pS-channel is highly selective to $K^+$.

The 10 pS-channel was also observed in cell-attached patches (21 out of 29 patches), but simultaneous activation of inwardly rectifying $K^+$ channels ($i_{krec}$; KAMEYAMA et al., 1983) and basal activity of muscarinic $K^+$ channels ($i_{kach}$; SOEJIMA and NOMA, 1984) often hampered the isolation of the 10 pS-channel in the cell-attached mode. This analytical problem could be overcome by the cell-free patch configuration, where $i_{krec}$ and $i_{kach}$ ran down much more rapidly than the 10 pS-channel. We, therefore, employed the inside-out condition in most experiments.

To characterize further the kinetic properties of the 10 pS-channel current, open- and closed-time histograms were constructed from original current traces that contained no smaller conductance channel openings. Figure 3 depicts representative results at $V_r$ of $-100$ mV from a holding potential of $+20$ mV. The distribution of burst durations was well fitted with a single exponential function, yielding one mean open time (A). In contrast, the closed-time histogram fitted two
Fig. 4. Openings of 3pS-channels. Original single-channel current traces containing the smaller conductance channel alone, measured from an inside-out patch at \( V_i \) of \(-80 \) (A) and \(-100 \) mV (C). Ensemble currents (B and D) were obtained from 61 and 40 data. Time and current calibrations are given to the bottom of each column. Vertical bars indicate 1 pA in A and C and 0.1 pA in B and D. Unitary current-voltage relationships (E) at external K\(^+\) concentrations of 150 mM (filled squares) and of 300 mM (open squares).

exponentials with a fast and a slow time constant: one derived from interburst (B) and another from intraburst closings (C).

**Smaller conductance channel**

In addition to the 10pS-channel, another channel activity with smaller conductance could be identified as already noted in Fig. 2. The life time of this channel was much longer than that of the 10 pS-channel. In Fig. 4A and C, original traces that contained no simultaneous openings of the 10 pS-channel are illustrated. Their ensemble average currents also showed slow deactivation (B, D). The unitary current-voltage relationship (E, filled squares) gave the single channel conductance of 3 ± 0.5 pS (\( n = 6 \)), and the reversal potential of 0 mV at 150 mM\([K^+]_o\). At 300 mM\([K^+]_o\) the single channel conductance and the reversal potential were 5 ± 0.5 pS and 15 ± 3 mV (\( n = 5 \), open squares), respectively. The results indicate that the 3 pS-channel is also selective to K\(^+\).

Because of multiple openings of the 3pS-channel (the maximal number of channels was usually >2) and of its small unit amplitude, we failed to conduct the distribution analyses of open and closed times of this smaller conductance channel.
Fig. 5. Demonstration of activation gate. A: three ensemble average currents obtained from traces recorded in a single patch at \( V_i \) of \(-80 \) mV from \( V_h \) of \(-60 \) mV (75 traces), \(-20 \) mV (90 traces) and \(+20 \) mV (54 traces). Hyperpolarizing voltage (\( V_i \)) pulses were applied every 2 s from various holding potentials (\( V_h \)) as shown in the inset of panel B. Arrows and smooth lines indicate the zero current level. B: peak amplitudes of inward tail currents (\( i_p \)) were normalized as a fraction of the maximal peak amplitude (\( i_{p,\text{max}} \)) and are plotted against \( V_h \). Smooth line was drawn by an equation: \( \frac{i_p}{i_{p,\text{max}}} = \frac{1}{1 + \exp (V_{0,5} - V_h) / s} \).

**Gating mechanisms determined from ensemble average currents**

How do these channels contribute to the deactivation process of whole-cell currents? With applying more positive \( V_h \) in the cell-attached mode, the opening probability increased in both 10pS- and 3pS-channels and their ensemble currents became larger (Fig. 5A). These results indicate the presence of an activation gate, compatible with that noted in whole-cell delayed rectifier currents (Noma and Irisawa, 1976; MacDonald and Trautwein, 1978; Shibasaki, 1987). We calculated the opening probability as the fraction of peak ensemble currents (\( i_p/i_{p,\text{max}} \)) and plotted it as the function of \( V_h \). In the experiment of Fig. 5, the activation curve was well fitted by a Boltzmann's equation (Hodgkin and Huxley, 1952):

\[
\frac{i_p}{i_{p,\text{max}}} = \frac{1}{1 + \exp (V_{0,5} - V_h) / s}^{-1},
\]

where a half activation potential (\( V_{0,5} \)) was \(-18 \) mV and a slope factor (\( s \))
Fig. 6. Deactivation of 10pS- and 3pS-channel currents. A: semilogarithmic plots of ensemble currents at $V_r = -80$ and $-100$ mV, the same currents as those shown in Fig. 2B and D. Upper traces represent the fitting for slower components, and lower traces the fitting for fast components. Slopes of smooth lines give time constants for decaying currents as indicated in the graph. Time calibration is given in the panel B. B: semilogarithmic plots of ensemble currents of the 3 pS-channel shown in Fig. 4B and D. They were fitted by a single exponential. Time constants thus estimated are indicated.

$11$ mV (Fig. 5B). In 4 experiments, $V_{0.5}$ was $-15 \pm 5$ mV and $s$ was $11 \pm 3$ mV. It is concluded that these channels are closed at about $-60$ mV and fully open at about $+20$ mV.

Two channels give different time courses of deactivation

Current data containing both 10 pS- and 3 pS-channel activities were employed to obtain the activation gate because of difficulty in discriminating the 3 pS-channel in the cell-attached condition. Using current data recorded in the cell-free condition, we tried to separate time courses for deactivation of these channels. Figure 6A depicts the plot of ensemble tail currents of 10 pS-channel on a semilogarithmic scale. The changes in tail currents were expressed by a sum of two exponential functions. In the other 3 experiments, ensemble tails gave two time constants of similar range, which corresponded well to those obtained in whole-cell tail currents (Fig. 1). Both time constants for the fast and slow deactivation became smaller at more negative $V_r$. 

*Japanese Journal of Physiology*
Semilogarithmic plots of ensemble tail currents of the 3 pS-channel also revealed the deactivation process, but in a single exponential manner (Fig. 6B). The time constant decreased with the larger hyperpolarization (1,200 ms at $V_e$ of $-80$ mV and 419 ms at $-100$ mV), and coincided with larger time constants from the data containing both channels (Fig. 6A: 1,494 ms at $-80$ mV and 551 ms at $-100$ mV). The 3 pS-channel appeared responsible for the slowly-decaying current. Thus, it is concluded that there are two independent deactivation processes mediated by two types of $K^+$ channels.

DISCUSSION

Two types of $K^+$-selective channels carrying delayed rectifier $K^+$ currents in the atrial cell membrane. In the mammalian atrial cell, the present study first showed co-existence of two types of channels having activation gates of different kinetics. These two channels may correspond to delayed rectifier $K^+$ currents identified by multicellular and whole-cell voltage clamp experiments, which is supported by substantial evidence as follows: 1) Both the 3 pS- and 10 pS-channels appeared to be $K^+$-selective. 2) They showed independent deactivation kinetics. 3) Time constants estimated from ensemble currents (81 ms and 551 ms as $-100$ mV) were similar to those in whole-cell experiments (96 ms and 463 ms at $-30$ mV), although direct comparison is impossible because of the difference in $[K^+]_o$. 4) These time constants also ranged within those recorded in a variety of cardiac preparations at the macroscopic current level, including sheep cardiac Purkinje fibers (NOBLE and TSIEN, 1969), frog atrium (OJEDA and ROUGIER, 1974), feline ventricle (MCDONALD and TRAUTWEIN, 1978), rabbit S-A node cells (DI FRANCESCO et al., 1979), and rabbit A-V node cells (KOKUBUN et al., 1982).

Single channel properties of atrial delayed rectifier channels: Comparison with previous studies. Single channel kinetics of rabbit nodal delayed rectifier $K^+$ has extensively been examined by SHIBASAKI (1987). He reported a single class of $K^+$ selective channels, having a mono-exponential distribution for open-times and a double exponential for the closed-times. Thus, the single channel kinetics and its conductance (11 pS at 150 mM $[K^+]_o$) was similar to those of the larger conductance channel in this study (10 pS). In cat ventricular (MCDONALD and TRAUTWEIN, 1978) and rabbit nodal preparations (NOMA and IRISAWA, 1976; SHIBASAKI, 1987), tail current analyses demonstrated an activation gate with similar voltage-dependence to that shown in the present study (Fig. 5).

During single channel studies on the nodal delayed rectifier, SHIBASAKI (1987) showed a "hook" in ensemble currents decaying upon repolarization after stronger depolarization which was attributed to the fast removal of the inactivation gate. In the present study, when $V_h$ was $+10$ or $+20$ mV, we also observed such a small initial increase in ensemble currents (e.g., Fig. 2B, D). Since no obvious "hook" phenomena in the ensemble currents calculated from 3 pS-channel activities alone were found (e.g., Fig. 4B, D), such an inactivation process appears unique in the
larger conductance channel. However, more detailed analyses would be necessary to further clarify the inactivation gating mechanism in these two classes of channels.

Single channel properties of delayed rectifier channels have widely been studied in other preparations, including squid axons (Conti and Neher, 1980), PCC4 embryonal carcinoma cells (Ebihara and Speers, 1984), hippocampal neurons (Rogawski, 1986), PC12 phenochromocytoma cells (Hoshi and Aldrich, 1984), and artificial reconstituted membrane (Coronado et al., 1984). In natural membranes, the delayed rectifier displays a conductance of 10–20 pS with bursting kinetics and a mean open time of <30 ms. These single channel properties are quite similar to those of the 10 pS-channel identified here.

Single channel conductance properties: Comparison with other cardiac K$^+$ channels. When compared to other cardiac K$^+$ channels that open at the plateau potential level, the inward rectification is one of the remarkable properties of both 10 pS- and 3 pS-channels. The inward rectification remained even in the absence of internal Mg$^{2+}$ ions, indicating that the channels differ from other cardiac inwardly-rectifying K$^+$ channels, where outward currents through the channels are blocked by internal Mg$^{2+}$ ions at their physiological concentrations (ATP-sensitive K$^+$ channels: Horie et al., 1987; inward-rectifier K$^+$ channels: Matsuda et al., 1987; muscarinic K$^+$ channels: Horie and Irisawa, 1989).

More recently, two types of K$^+$ channels, exhibiting the delayed rectifying property, were found in avian embryonic heart cell membranes (62 and 15 pS, external K$^+$: 4 mM; internal K$^+$: 145 mM; Clapham and Logothetis, 1988). Because these channels had ohmic unitary conductances, they are different from the channels noted in this study. For the same reason, a 14 pS-K$^+$ channel in guinea pig ventricular cell membranes ($i_{K_p}$; Yue and Marban, 1988) differs, because $i_{K_p}$ showed a quite rapid activation on depolarization and no apparent deactivation on repolarization without apparent rectifying properties. Furthermore, the conductance of $i_{K_p}$ appears insensitive to changes in [K$^+$]$_o$, whereas those of 10 pS- and 3 pS-channels were increased by raising [K$^+$]$_o$.

Since recent whole-cell clamp studies have focused upon the regulation of these K$^+$ currents by neurotransmitters such as norepinephrine, isoprenaline and acetylcholine and their signal transduction pathways (Hume, 1985; Kameyama et al., 1986; Bennett and Begenisich, 1987) the single channel analyses will offer a more promising approach to further elucidate underlying molecular mechanism(s).

The encouragement and support by Dr. M. Kuno during the work are gratefully acknowledged. The authors thank Drs. H. Irisawa and Y. Okada for critically reading the manuscript and Dr. M. Komori for providing computer analysis programs. This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan, and Research Grants from the Japan Cardiovascular Research Foundation and the Japan Heart Foundation.
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