Resting K Conductances in Pacemaker and Non-pacemaker Heart Cells of the Rabbit

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Abstract Currents through the inward rectifier K channel (i_K_rec) and the ACh-operated K channel (i_K_ACh) were recorded in isolated heart cells of rabbit using the patch clamp technique with electrodes having 0.5-1 μm tip inner diameter. The maximum number of overlaps of open i_K_rec channels per patch was measured over 347 experiments. An average of 2.3 was found in ventricular cells and 0.03-0.06 in sinoatrial (S-A) and atrioventricular (A-V) node cells. The estimated total number of the i_K_rec channels for each ventricular cell was great enough to supply the resting K conductance of the cell. The i_K_ACh channel was present in S-A and A-V node cells, but was never observed in the ventricular cells. The resting conductance of the nodal cells measured with whole cell clamp recordings was about 16 times smaller than that of the ventricular cells, and was hardly decreased at all by the removal of K+ from the bath solution. Thus, the lower membrane potential of the nodal cells compared with that of the ventricular cells was attributed to the smaller K conductance of the resting membrane, which is due to the very low density of the i_K_rec channel. On the other hand, the i_K_ACh channel, when activated by neural regulation, may play a major role in generating the resting K conductance of the nodal cells.

Key Words: single heart cell, pacemaker cell, ventricular cell, resting K conductance, inward rectifier K channel.

One of the major differences between pacemaker and non-pacemaker heart cells is that the resting potential of the former is less negative than that of the latter (for review, Irisawa, 1978; Brown, 1982). The resting potential of the nodal cells determined as the zero-current potential in the steady-state current voltage relation is about -40 mV (Noma and Irisawa, 1975; Noma et al., 1980). Because of this low resting potential, the deactivation of the delayed outward K current results in pacemaker depolarization (Dudel and Trautwein, 1958). This low
resting potential of nodal cells, has so far been explained only in terms of the large ratio of membrane permeability to Na⁺ compared to that for K⁺ (P₉/P₆). In the present paper, the ionic channels activated in the resting potential range were examined in nodal and ventricular cells by the patch clamp technique (Hamill et al., 1981), which allowed the Na and K conductances to be separated, using the patch electrode being filled with Na⁺ or K⁺ solutions. We found a marked difference between the nodal cells and the ventricular cells in the distribution of K channels. This difference was well correlated with the macroscopic current recordings in these two kinds of cardiac cells, and seemed to explain the resting potentials in each cell.

METHODS

The method to prepare single cells is the same as that described elsewhere (Taniguchi et al., 1981). Briefly, rabbit hearts were perfused through the coronary artery with 0.04% collagenase dissolved in Ca-free Tyrode solution (Powell and Twist, 1976; Isenberg and Klöckner, 1982). After enzymatic treatment, a small piece of tissue was dissected out from the S-A node, A-V node, or the ventricle and was shaken or teased in the recording chamber to isolate single cells. Ventricular cells of rod shape and nodal cells of round shape were used in the experiment. The spontaneously beating nodal cells were made quiescent by adding 2×10⁻⁷ M ACh to the bath before the patch electrode was applied. The resting potential was more negative than the zero-current potential by about 29 mV in the voltage clamp experiment (Sakmann et al., 1983). The composition of normal Tyrode solution was, in mM: NaCl, 142.0; KCl, 5.4; CaCl₂, 1.8; MgCl₂, 0.5; NaH₂PO₄, 0.3; glucose, 5.5; and HEPES-NaOH buffer, 5 (pH=7.4). In K-free Tyrode solution, 5.4 mM K was substituted for equimolar Na. In K- and Na-free Tyrode solution, Tris was used for the replacement of both ions.

The patch clamp technique was essentially the same as described by Hamill et al. (1981). The heat-polished glass pipettes with tips of 0.5–1.0 μm inner diameter were filled with 150 mM KCl or 150 mM NaCl solution buffered with 10 mM HEPES to pH=7.4. The electrode resistances were 5–10 MΩ. The single channel current was recorded on the magnetic tape for later analysis using a computer (Hitachi, E 600).

In the whole-cell recording of the membrane current, a feedback resistor of 100 MΩ instead of 10 GΩ was used, and electrodes of larger tip (3–4 μm in diameter) were used. The pipette solution was continuously perfused by attaching a special device to the suction pipette, which is described in detail elsewhere (Soejima and Noma, 1983). A thin polyethylene inlet tubing was inserted into the suction pipette, and the test solution was led off from one of the small reservoirs to the electrode tip using a negative pressure of −20 to −40 cmH₂O. The control pipette solution contained, in mM, K aspartate, 110; KCl, 20; MgCl₂, 1; ATP (K salt, Sigma or Na salt, Yamasa), 5; EGTA, 1; and HEPES-KOH buffer, 5 (K-rich Japanese Journal of Physiology
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solution). In some experiments, equimolar Cs was substituted for K (Cs-rich solution). Ventricular cells smaller than 100 × 30 μm in size were used to ensure effective space clamp. The electronic apparatus used for the whole-cell clamp is described elsewhere (OHARA et al., 1983).

RESULTS

Single K-channel currents at the resting potential

In neither the ventricular nor the nodal cells did the electrode containing 150 mM NaCl solution record significant channel activity in the steady-state over the potential range of 20 mV± (resting potential) (Fig. 1A). Although single channel currents smaller than the background noise level (0.2–0.5 pA) might be missed with the present recording technique, the data may suggest a very small Na conductance of the resting membrane (see also Fig. 5 for the effect of

Fig. 1. Two types of single K+ channel currents in the heart (cell attached recordings). (A) The top trace was obtained from an A-V node cell with the electrode containing 150 mM NaCl instead of equimolar KCl in the pipette solution. In the lower part, recordings of the current through the \( i_{K,rec} \) channels in the ventricular cells (V) and those through the \( i_{K,ACH} \) channels in the S-A node cell (S-A) and the A-V node cell (A-V) were obtained using 150 mM KCl electrodes. The records were displayed through a low-pass filter at 1 kHz of the cut-off frequency. In the ventricular cell, the overlaps of two open channels are illustrated. Because of the low-pass filter, the shorter events of \( i_{K,ACH} \) channel were displayed on a smaller scale than in actuality. The unit amplitude of the current was measured from the longer events. (B) The number of the \( i_{K,rec} \) channels within a membrane patch was measured as the maximum number of overlapping open channels observed in a continuous recording for more than 5 min. This approximation was taken simply because it was easier than estimating the total number of channels by the least-squares fit of the binomial distribution to the amplitude histogram. The average of channel number in the ventricular cell, S-A node cell, and A-V node cell are shown in the graph. \( n \) indicates the number of experiments.

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removal of Na+ on the whole-cell current).

On the other hand, when we applied the 150 mM KCl electrode, large inward-going single channel currents were observed in both ventricular and nodal cells. The currents were separated into two groups; one of which was observed most frequently in the ventricular cells (Fig. 1, V), while the other appeared in the nodal cells (Fig. 1, S-A and A-V) and never in the ventricular cells. In the atrial cells, both types of currents were recorded. One of them was attributed to the inward rectifier K channels in the ventricular cells (TRUBE et al., 1981; KAMEYAMA et al., 1983) and the other to the ACh-operated K channels in the atrial and nodal cells (SAKMANN et al., 1983; SOEJIMA and NOMA, 1983). In agreement with the previous studies, the open time of the latter channel was much shorter (life-time \( \leq 0.5-1.5 \) msec) than that of the former (50-100 msec). The single channel conductance of the former type of channel (about 45 pS with 150 mM K) was smaller than that of the latter channel (about 55 pS). Both types of current were recorded when Cl ions in the pipette were replaced by aspartate ions, and the reversal potential of the two currents was always at about the K equilibrium potential.

The inward rectifier K channels \( (i_{K_{rec}}) \) were rarely observed in the nodal cells. The traces shown in Fig. 2 are such examples for the A-V node cell. When the patch membrane was hyperpolarized, the amplitude of the current increased and the open time of the channel decreased. At the potentials positive to the reversal potential, no significant outward-going channel current was recorded (not shown). The slope conductance of 45.5 pS (at 150 mM K+) agreed well with those observed with the \( i_{K_{rec}} \) in the ventricular cells (40-50 pS).

The contribution of the above K channels to the membrane K conductances at the resting potential can be estimated by calculating the time-averaged current flow through all the K channels within a patch membrane (mean patch current). The mean patch current was calculated by integrating the current flow during channel open periods and dividing the integral by the total time for the sample (48-80 sec). The small variation in the size of the membrane patch was neglected when averaging large numbers of experiments using electrodes of similar tip size (about 1.5 \( \mu \)m inner diameter). The patch current of 1.74±1.18 pA (mean ±S.D., \( n=18 \)) in the ventricular cells was much larger than 0.09±0.09 pA (\( n=41 \)) in the A-V node cells or 0.05±0.06 pA (\( n=24 \)) in the S-A node cells. Since the current flow through the \( i_{K_{rec}} \) channel is much larger than that due to the spontaneous openings of the \( i_{K_{ACh}} \) channel, the differences in the distribution of the \( i_{K_{rec}} \) channel among the different cells were examined as shown in Fig. 1B. As the first approximation, the maximum number of overlaps of open channels during a continuous recording of more than 5 min was taken as the number of available channels within a given patch. The average number of the \( i_{K_{rec}} \) channels in the ventricular cells (2.32, \( n=71 \)) was more than 10 times larger than that in the nodal cells. The average number of \( i_{K_{rec}} \) channels was 0.03 (\( n=39 \)) in the S-A node cell and 0.06 (\( n=237 \)) in the A-V node cell.

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Although the above findings are limited to relatively large single channel currents at the very high external K concentration of 150 mM, they suggest a very low value of $P_K$ as the main cause of the larger $P_{Na}/P_K$ ratio in the nodal cell when compared with the ventricular cell. If this is true, the input resistance should be much higher in the nodal cell than in the ventricular cell. Figure 3A compares the membrane currents of the whole cell in response to hyperpolarizing pulses in 10 mV steps from the holding potential of −40 mV. In the S-A node cell, the initial jumps of current were very small and were followed by the time-dependent development of the hyperpolarization-activated current. In the ventricular cells, on the other hand, relatively large current jumps were followed by a slight decay of the

![Graph showing current-voltage relationship](image-url)
current, which soon reached a steady level. In order to compare the amplitude of the current through a given area of surface of the cell, the surface area of ventricular cells was approximated as being twice as large as the area of the cells as judged from photographs. The surface area of nodal cells was estimated by assuming the cell shape to be a plane oblate spheroid. The amplitude per unit membrane was plotted in Fig. 3B for the initial current (5–10 msec) and steady-state current (1 sec) against the membrane potentials. The membrane resistance at the zero current potential (about −40 mV) in 14 nodal cells was 12.0 ± 2.0 kΩ·cm² and it was much higher than that of 0.76 ± 0.17 kΩ·cm² at about −70 mV in 8 ventricular cells.
Effects of removing $K^+$ and $Na^+$ on the nodal membrane currents

The contributions of $K^+$ and $Na^+$ conductances to the resting membrane of the A-V node cells were tested by examining effects of removing $K^+$ and $Na^+$ from the normal Tyrode solution and by removal of $K^+$ from the pipette solution. The membrane currents in response to various voltage clamp pulses were first recorded with K-rich pipette solution in the normal Tyrode solution (left column in Fig. 4). $K^+$ was then removed from both the bath and the pipette solutions. Ten minutes after switching the bath and pipette solutions, the outward current tail on repolarization disappeared (Fig. 4B, upper recordings). The hyperpolarization-activated current also disappeared (lower recordings). However, the change in the amplitude of the initial current on hyperpolarizations was quite small, when compared before and after $K^+$ removal.

Figure 5 shows the $I-V$ curves measured from the initial current (5-10 msec) and at 1 sec after the onset of the clamp pulses. The closed circles indicate the control $I-V$ curves and triangles after the removal of $K^+$ from both the bath and

![Fig. 4. Effects of removing K on the membrane currents in the single A-V node cell. In A, the bath solution was the normal Tyrode and the pipette solution was 140 mM K-aspartate. In B, the membrane currents carried by $K^+$ were blocked by switching the bath solution to K-free Tyrode and the pipette solution to 140 mM Cs-aspartate. The holding potential was $-30\text{ mV}$ and the test pulse duration was 1 sec. In the upper traces of A and B, the currents in response to depolarizing clamp pulses to $-10$, $20$, and $50\text{ mV}$ are superimposed, and in the lower traces, those in response to hyperpolarizations to $-50$, $-70$, and $-90\text{ mV}$. In each panel, the upper traces show the voltage recordings and the lower traces the current recordings. On the left of each current trace, zero current level is indicated.](image)
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The slope conductance of the initial current on hyperpolarization was not much affected by the removal of K+. The decrease of the slow inward current may be due to "run down" phenomenon observed in the dialyzed cells. Figure 5 also shows the effect of Na+ removal from the K-free bath solution in the same cell. The slope conductance on hyperpolarization in the initial current was scarcely affected. These findings confirmed that the K conductance in the resting membrane is extremely small in nodal cells.

DISCUSSION

The above findings are straightforward in indicating the lower K conductance of the nodal cells compared to the ventricular cells. In the latter, the conductance $g_{K}$ at 5.4 mM K produced by the total $i_{K-rec}$ channels per unit membrane area was estimated to compare with the resting conductance measured with whole-cell recordings. The $g_{K}$ is given as,

$$g_{K} = \gamma \cdot p \cdot N,$$

where $\gamma$ is the single channel conductance, $p$ the probability of the open state of the channel at the resting membrane potential, and $N$ the number of the $i_{K-rec}$ channels within a unit membrane. The value of $N$ was estimated from the average number of $i_{K-rec}$ channels within a membrane patch. Assuming the diameter of the

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patch membrane to be 1–2 μm, \( N \) is 29.5–7.4 × 10^7 cm\(^{-2}\). The value of \( p \) measured in ventricular cells (Kameyama et al., 1983) was about 0.9 at potentials close to the reversal potential and the slope conductance of the \( i_{K,rec} \) channel was proportional to the square root of \( K^+ \) concentration and about 45 pS at 150 mM K on the outer side of the membrane over the potential range negative to the reversal potential. Thus, \( \gamma \) of 8.54 pS was assumed at 5.4 mM K. This value of \( \gamma \) is still for the inward-going current, but it may be assumed that the outward-going conductance is close to this value at potential ranges between -90 to -70 mV. This is because the rectification of the channel conductance becomes less obvious with decreasing \( K^+ \) concentration. Thus, the \( g_K = 22.7 - 5.7 \times 10^{-4} \) S cm\(^{-2}\), or the resistance is 440–1,760 \( \Omega \) cm\(^{-2}\). This value well agrees with the input resistance of 760 \( \Omega \) cm\(^{-2}\) in the present experiment. Thus, it may be concluded that the resting K conductance of the ventricular cell is mainly supplied by the \( i_{K,TeC} \) channels.

The \( i_{K,ACh} \) channel instead of the \( i_{K,rec} \) channel may be a major source of the resting K conductance in the nodal cells. In the absence of ACh, the resting conductance and the amplitude of the time-averaged current through the patch membrane was about 20 times smaller in the nodal cells than in the ventricular cells. This small resting conductance was only slightly decreased by removing \( K^+ \) from both the bath and the pipette solutions. Although in the nodal cells, \( i_{K,ACh} \) channels open even in the absence of ACh (Sakmann et al., 1983; Soejima and Noma, 1983) and the nodal cells have a very small number of \( i_{K,rec} \) channels, the above findings indicate that the contribution of these K channels to the total membrane conductance at around -40 mV is negligibly small in the nodal cells. It must be noted that in generating the pacemaker depolarization the deactivation of \( i_K \) during the diastolic period should be very effective as against the background of low resting K conductance. In the intact tissue, the concentration of ACh may be increased when the vagal activity increases. Then the resting conductance provided by \( i_{K,ACh} \) channel increases and decelerates the spontaneous rhythm of the pacemaker cells by hyperpolarizing the membrane.

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