Heterogeneous distribution of the two components of delayed rectifier $K^+$ current: a potential mechanism of the proarrhythmic effects of methanesulfonanilide class III agents

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Abstract

Objective: To elucidate the regional difference of the $K^+$ current blocking effects of methanesulfonanilide class III agents.

Methods: Regional differences in action potential duration (APD) and E-4031-sensitive component ($I_{Kr}$) as well as $I_{Kr}$-insensitive component ($I_{Kr}$o) of the delayed rectifier $K^+$ current ($I_{Kr}$) were investigated in enzymatically isolated myocytes from apical and basal regions of the rabbit left ventricle using the whole-cell clamp technique.

Results: At 1 Hz stimulation, APD was significantly longer in the apex than in the base (223.1 ± 10.6 vs. 182.7 ± 14.5 ms, $p<0.05$); application of 1 μM E-4031 caused more significant APD prolongation in the apex than in the base (32.5 ± 6.4% vs. 21.0 ± 8.8%, $p<0.05$), resulting in an augmentation of regional dispersion of APD. In response to a 3-s depolarization pulse to $+40$ mV from a holding potential of $-250$ mV, both $I_{Kr}$ tail and $I_{Kr}$ tail densities were significantly smaller in apical than in basal myocytes ($I_{Kr}$: 1.56 ± 0.13 vs. 2.09 ± 0.21 pA/pF, $p<0.05$; $I_{Kr}$: 0.40 ± 0.15 vs. 1.43 ± 0.23, $p<0.01$), whereas $I_{Kr}$ tail density was significantly greater in the apex than in the base (1.15 ± 0.13 vs. 0.66 ± 0.11 pA/pF, $p<0.01$). The ratio of $I_{Kr}$/$I_{Kr}$ for the tail current in the apex was significantly smaller than that in the base (0.51 ± 0.21 vs. 3.09 ± 0.89; $p<0.05$). No statistical difference was observed in the voltage dependence as well as activation and deactivation kinetics of $I_{Kr}$ and $I_{Kr}$ between the apex and base. Isoproterenol (1 μM) increased the time-dependent outward current of $I_{Kr}$ by 111 ± 8% during the 3-s depolarizing step at $+40$ mV and its tail current by 120 ± 9% on repolarization to the holding potential of $-50$ mV, whereas it did not affect $I_{Kr}$.

Conclusions: The regional differences in $I_{Kr}$, in particular differences in its two components may underlie the regional disparity in APD, and that methanesulfonanilide class III antiarrhythmic agents such as E-4031 may cause a greater spatial inhomogeneity of ventricular repolarization, leading to re-entrant arrhythmias. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Experimental; Heart; Electrophysiology; K-channel; Action potential; Antiarrhythmic agents; Myocytes

This article is referred to in the Editorial by A.C.G. van Ginneken and M.W. Veldkamp (pages 20–22) in this issue.

1. Introduction

Most of newly developed class III antiarrhythmic agents (e.g., d-sotalol, E-4031, dofetilide) exert their antiarrhythmic effects by selectively inhibiting the rapidly activating component of the delayed rectifier $K^+$ current ($I_{Kr}$) [1]. However, they possess a common unfavorable feature: their action potential duration (APD) prolonging effect diminishes at high stimulation frequencies. This frequency-dependent variation in efficacy might reduce their usefulness in terminating tachyarrhythmias and even cause proarrhythmic effects [2]. These agents can produce marked QT interval prolongation with resultant polymorphic ventricular tachycardia including the torsade de pointes syndrome [2,3]. The mechanism contributing to the drug-
induced torsade de pointes include early afterdepolarization and regional dispersion of repolarization [4,5]. The former would lead to local triggered activity, and the latter would set the stage for reentry by producing a critical spatial inhomogeneity of refractoriness [4,5].

In the ventricle of many species (e.g. dog, guinea pig, human), there is a gradient of action potential configuration and duration across the ventricular wall [6–10]. In addition, a distinctive group of cells (M cells), located in deep subepicardial to midmyocardial region, has been identified [11]. These cells are reported to have the electrical property that their APD prolongs markedly with slow stimulation, especially during ischemia or after exposure to antiarrhythmic drugs, perhaps because the slowly activating component of \(I_K\) (\(I_{\text{Kr}}\)) is expressed at very low levels in these cells [7]. At slow rates, action potentials in these cells not only prolong markedly, they may also display discontinuities in repolarization (early afterdepolarizations), which are thought to be involved in the genesis of long QT-related arrhythmias.

The rabbit ventricular myocytes has been regarded to be a suitable preparation for the evaluation of class III effects, because a variety of voltage-gated K⁺ channels exist which resemble those in human cardiac cells [12–18]. It was also reported that many class III drugs easily induce torsade de pointes in rabbits [19]. The existence of only one component (\(I_{\text{Kr}}\)) of \(I_K\) in rabbit ventricular myocytes [15,20–22] might be the underlying mechanisms. However, Salata et al. recently demonstrated the existence of \(I_{\text{Kn}}\) in rabbit ventricular myocytes [23]. In isolated Langendorff-perfused rabbit heart, our previous report showed that the QT interval of cardiac surface electrograms (measured as the interval from the initial sharp negative deflection of the QRS complex to the peak of T-wave), which reflects the action potential duration at the recording site, was significantly longer in the apical than the basal region [24]. Furthermore, this regional difference in APD was augmented very much by the application of class III antiarrhythmic agent E-4031 or sotalol [24]. These findings suggest the existence of regional differences in the density of \(I_K\) and its two components (\(I_{\text{Kn}}\) and \(I_{\text{Kr}}\)) in rabbit ventricles, contributing to the above-mentioned electrical heterogeneity. In the present study, we demonstrated a significant heterogeneity of \(I_K\) and its two components (\(I_{\text{Kn}}\) and \(I_{\text{Kr}}\)), and indicated that such heterogeneity is important in understanding the physiological function of \(I_K\) as well as the pharmacological and clinical significance of \(I_K\) blockers.

2. Materials and methods

2.1. Myocyte isolation

This investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Japanese white rabbits of either sex weighing 1.5 to 2.0 kg were anesthetized with thiopental sodium after being heparinized, and the hearts were rapidly excised and mounted via the aorta on a Langendorff retrograde perfusion apparatus. The hearts were first perfused with normal Tyrode’s solution (gassed with 100% O₂ at 37°C) for 3–5 min, secondly with calcium-free Tyrode’s solution for 10–15 min, and finally with 0.12 mg/ml collagenase (Yakult, Japan) containing calcium-free Tyrode’s solution for 15 min. The hearts were subsequently perfused with high-potassium storage solution (KB solution) for 5 min. After the perfusion, the left ventricle was cut perpendicularly to the apex/base axis into three blocks: apex, middle and base. The apical block was about 5 mm thick measured from the apex of the heart, and the basal block was of the same thickness from the atrioventricular groove. The apical and basal muscle masses served for cell isolation and the remaining middle one was discarded. The apical and basal portions were placed separately in beakers containing KB solution and minced with a pair of surgical scissors. The supernatants were then passed through 200 µm stainless steel mesh, and the filtrates were washed two times with KB solution by centrifuging at a speed of 1000 rpm for 5 min. The cells were stored in KB solution at 4°C before use.

2.2. Electrophysiological recordings

The standard whole-cell patch-clamp method was used for recording the membrane potential and current with a patch-clamp amplifier (List-medical, Darmstadt, Germany). An aliquot of the cell suspension was placed in the recording chamber on the stage of an inverted microscope (Diaphoto, Nikon Co., Tokyo). A brief period was allowed for cell adhesion to the coverslip at the bottom of the chamber, and then the cells were superfused with normal Tyrode’s solution at 3 ml/min. The bath temperature in all experiments was maintained at 34°C. The suction pipette of borosilicate glass has a resistance of 3–5 MΩ after filling with the pipette solution. The cell capacitance was obtained by calculating the area under the capacitive transient evoked by a small voltage step from a holding potential of −50 mV. The cell capacitance and series resistance were electrically compensated by about 50–70%. Command potentials were generated by a multichannel stimulator (Nihon-Kohden, Tokyo, Japan). Action potential was recorded under whole-cell current-clamp mode, and was elicited by injecting 5-ms-long rectangular pulses of depolarizing current through the pipette. Voltage signals were displayed on an oscilloscope (Tektronix, 5111A, USA) and photographed. Current signals were
filtered at 1 kHz, and stored on a personal computer (NEC9801DA Tokyo, Japan) for later analysis.

2.3. Solutions and drugs

Tyrode’s solution for cell isolation and the recording of action potential was composed of (in mM): NaCl, 143; KCl, 5.4; CaCl₂, 1.8; MgCl₂, 0.5; NaH₂PO₄, 0.25; HEPES, 5; and glucose, 5.6, pH adjusted to 7.4 with NaOH. The calcium-free Tyrode’s solution was the same as above except that it lacked CaCl₂. The KB solution contained (in mM): KOH, 82; l-glutamic acid, 50; KCl, 40; KH₂PO₄, 20; taurine, 20; HEPES, 10; MgCl₂, 3; glucose, 10; EGTA, 0.5, pH adjusted to 7.4 with KOH. The internal pipette solution contained (in mM): KOH, 60; KCl, 80; aspartate, 40; HEPES, 5; EGTA, 10; MgATP, 5; sodium creatinine phosphate, 5; and CaCl₂, 0.65 (pH 7.2; pCa 8.0). When the delayed rectifier K⁺ current was measured, the superfusate was changed to a solution (NMG solution) composed of the following (in mM): N-methyl-D-glucamine, 149; MgCl₂, 5; CaCl₂, 0.9; HEPES, 5 and nisoldipine, 0.003, pH adjusted to 7.4 with HCl. The two components of Iₖₑ, Iₖᵣ, and Iₖₛᵣ, were pharmacologically separated by the application of E-4031, a selective Iₖᵣ blocker, at a concentration of 5 μM. This concentration was reported to be enough to block the fast activating component completely [25]. Iₖₑ and Iₖᵣ were estimated by the E-4031-sensitive component and E-4031-insensitive component, respectively. The E-4031-insensitive component (Iₖₛᵣ) can be blocked by 30 μM chromanol 293B, a blocker of Iₖᵣ [26]. The E-4031-sensitive component (Iₖₑ) was obtained by digital subtraction of the E-4031-insensitive current (Iₖₛᵣ) from the control current (Iₑ). For comparison of currents derived from the two regions, current amplitudes were normalized to the cell capacitance.

Fig. 1. Effect of E-4031 (1 μM) on APD at 1 Hz stimulation in apical and basal myocytes. Upper panel: individual representative recordings of action potentials in control (open circle) and those after E-4031 (closed circle) in an apical (A) and a basal (B) myocyte. Lower panel: summarized data showing the regional difference in APD and changes due to E-4031 application. *p<0.05 and **p<0.01 vs. each control; †p<0.05 and ‡p<0.01 vs. apical values; n=7 in each group.

2.4. Statistical analysis

Data were expressed as mean±SE. The curve-fitting program Axograph 3 (Axon Instruments, Inc., USA) was used in data analysis. Statistical analysis was performed using paired or unpaired Student’s t-test.

3. Results

3.1. Action Potential Characteristics

We first studied the action potential in the apical and basal myocytes of the rabbit left ventricle. Action potentials were recorded in Tyrode’s solution at 1 Hz stimulation. The steady-state APD was determined after 5 min stabilization in control and then after 5 min application of E-4031 (1 μM). The data were obtained from cells showing beat-to-beat variation of APD less than 10%. Consecutive 5 APDs (measured at 90% repolarization) in each experimental condition were averaged and compared. Typical recordings from an apical and a basal myocyte before and after the application of E-4031 are shown in the upper panel of Fig. 1. APD was longer in the apical (228 ms) than basal myocyte (202 ms) in control. Application of 1 μM E-4031, a concentration which blocks about 50% of Iₖᵣ [25], prolonged APD more markedly in the apex (35%) than in the base (19%). However, the resting potential and...
action potential amplitude remained unchanged in both cell types. The summarized data on APD are shown in the lower panel of Fig. 1, where APD under the control condition was significantly longer in the apex than in the base (223.1±10.6 vs. 182.7±14.5 ms, p<0.05), and application of 1 μM E-4031 caused more significant APD prolongation in the apex than in the base (32.5±6.4% vs. 21.0±8.8%, p<0.05; n=7 in each group), resulting in an augmentation of regional dispersion of APD. These results are consistent with our previous data on the regional difference of repolarization estimated by surface potentials in Langendorff-perfused rabbit heart [24].

3.2. $I_K$ in Tyrode’s and NMG solutions

In rabbit ventricular myocytes, the tail of the delayed rectifier $K^+$ current ($I_{K1}$) is small in amplitude and easily masked by the large inward rectifier ($I_{K2}$). As a result, elimination of $I_{K1}$ is commonly required during voltage-clamp experiments to assess the pharmacological modulation of $I_K$ in ventricular myocytes. This goal was achieved by use of extracellular $K^+$-free solution in order to obtain a reliable recording of $I_K$ [27]. However, it was proposed that the rapidly activating component of $I_K$ was greatly suppressed in extracellular $K^+$-free condition in some animal species such as guinea pigs and dogs [7,28]. To study whether such features exist in rabbit ventricular myocytes or not, we comparatively investigated the current-voltage relationships of $I_{K1}$ in Tyrode’s solution (containing 3 μM nisoldipine) and NMG solution (Fig. 2). Both apical and basal myocytes were employed for the experiments. $I_K$ was elicited by applying depolarizing

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**Fig. 2.** Comparison of $I_{K1}$ in Tyrode’s and NMG solutions. $I_{K1}$ was elicited by applying depolarizing potentials to various levels ranging from $-40$ mV to $+60$ mV for 3 s from a holding potential of $-50$ mV. Nisoldipine (3 μM) and chromanol 293B (30 μM) were present throughout the experiments to block $I_{Na}$ and $I_{Ca}$, respectively. A: representative recordings obtained in Tyrode’s (left) and NMG (right) solutions. The current traces were from the same myocyte; the arrows indicate the zero current level. B: I-V curve of $I_{K1}$ obtained in Tyrode’s (open circle) and NMG (filled circle) solutions (n=7). Changing Tyrode’s solution to NMG solution significantly modified the time-dependent outward current (left), whereas had no significant effects on the tail current (right) of $I_{K1}$.
potentials to various levels ranging from −40 mV to +60 mV for 3 s from a holding potential of −50 mV. Chromanol 293B (30 µM) was present during the experiments to block the \( I_{K} \) component. Representative recordings obtained in Tyrode’s (left) and NMG (right) solutions are shown in Fig. 2A; the I–V curves of \( I_{K} \) (time-dependent outward current and tail current) obtained in Tyrode’s (open circle) and NMG (filled circle) solutions are presented in Fig. 2B. There were substantial differences in the time-dependent outward current between the two solutions: the current density at negative potentials (−40 to 20 mV) in NMG solution was significantly larger than that in Tyrode’s solution. In addition, the inward rectification of the current in NMG solution was less prominent than that in Tyrode’s solution. In contrast, there was no significant difference in the density of \( I_{K} \) tail current at all potentials. Therefore, in the following study, we mainly investigated the regional difference in the tail current of \( I_{K} \) and its two components.

3.3. \( I_{K} \) in apical and basal myocytes

To reveal the underlying ionic mechanisms of the above heterogeneities in APD, we studied the delayed rectifier (\( I_{K} \)), one of the most important repolarizing \( K^{+} \) current in rabbit ventricular myocytes, in the apical and basal regions. \( I_{K} \) was activated by applying voltage clamp steps for 3000 ms at 0.1 Hz from a holding potential of −50 mV to a range of depolarizing levels between −40 mV and +60 mV. Representative current tracings of \( I_{K} \) are shown for an apical (A) and a basal myocytes (B) in the upper panel of Fig. 3. Amplitudes of the time-dependent outward current during depolarization and the tail current on repolarization in apical myocytes were much smaller than those in basal myocytes. E-4031 (5 µM) reduced those currents of the apical myocyte markedly (by 90%), indicating that they are generated mainly by \( I_{K} \). On the contrary, E-4031 decreased \( I_{K} \) of the basal myocyte only slightly (by 10%), indicating that it is mainly due to \( I_{Ks} \).

The lower panel of Fig. 3 illustrates the individual data of the \( I_{K} \) tail density following a depolarization to +40 mV. \( I_{K} \) tail current was divided into E-4031-sensitive (\( I_{Ks} \)) and -insensitive (\( I_{Ki} \)) components. There was a large cell-to-cell variation in the amount of \( I_{Ks} \); \( I_{Ki} \) was not detectable in three of nine apical cells. The density of \( I_{K} \) tail was significantly smaller in the apical than in the basal myocytes (1.56±0.13 vs. 2.09±0.21 pA/pF; \( p<0.05 \)).

As to the E-4031-sensitive component (\( I_{Ks} \)), the tail current density of \( I_{Ks} \) in the apex was approximately twice that in the basal myocytes (1.15±0.13 vs. 0.66±0.11 pA/pF; \( p<0.01 \)). In contrast, the tail current density of E-4031-insensitive component (\( I_{Ki} \)) in the apex was only about one third of that in basal myocytes (0.40±0.15 vs. 1.43±0.23, \( p<0.01 \)). The ratio of \( I_{Ki}/I_{Ks} \) for the tail current in the apex was significantly smaller than that in the base (0.51±0.21 vs. 3.09±0.89; \( n=9 \), \( p<0.05 \)).

3.4. Voltage-dependent activation of \( I_{K} \)

One possible explanation for the regional differences in \( I_{K} \) is that the voltage dependence of its activation is different in the two cell types. To determine whether any difference in voltage-dependent activation properties is present between the apical and basal myocytes, I–V relationships for \( I_{K}, I_{Ks} \) and \( I_{Ki} \) of both regions were examined by applying voltage clamp steps for 3 s from a holding potential of −50 mV to different depolarizing levels ranging from −40 + to +60 mV at 0.1 Hz (Fig. 4).

Fig. 4A and B illustrate the current-voltage relationship of the tail current of \( I_{Ks}, I_{Ki} \) and \( I_{Ki} \) of the two regions. In apical myocytes (A), the amplitudes of \( I_{K} \) and \( I_{Ki} \) tail currents increased in parallel with depolarization, reaching saturation at about +20 and +10 mV. In basal myocytes (B), the amplitudes of \( I_{K} \) and \( I_{Ki} \) tail currents increased with depolarization in similar voltage-dependent manners, and saturated at potentials of +40 and +50 mV, respectively. \( I_{Ks} \) component of basal myocytes reached saturation at +10 mV. This is consistent with that in apical myocytes.

In Fig. 4C and D, the tail current amplitudes have been normalized to the current amplitude at the most positive potential, and the relative activation curves were drawn by fitting the relative tail current amplitude as a function of test potential to the Boltzmann equation

\[
\frac{I_{K}}{I_{max}} = \frac{1}{1 + \exp(V_{1/2} - V_{i}/k)}
\]

where \( V_{1/2} \) is the voltage at which half activation is achieved, \( V_{i} \) is the test potential, and \( k \) is the slope factor. The voltage dependent activation parameters for \( I_{Ks}, I_{Ki} \) and \( I_{K} \) were not significantly different between the apical and basal myocytes (see Fig. 4), indicating that the voltage dependent activation properties of \( I_{Ks}, I_{Ki} \) and \( I_{K} \) are identical in the two regions.

3.5. Time-dependent activation of \( I_{K} \)

Fig. 5 illustrates the results of envelope-of-tails tests performed in the two cell types. Envelopes of the \( I_{K} \) tail current were evoked by applying depolarizing pulses to +40 mV at durations ranging from 100 to 4000 ms from a holding potential of −50 mV and tail currents after each pulse were measured on return to the holding potential. Upper panels show representative tracings of an apical and a basal myocyte, respectively. Lower panels show the averaged time courses of tail envelopes obtained by fitting the tail current amplitude to a single exponential function of the pulse duration. The relative contributions of \( I_{Ks} \) and \( I_{Ki} \) to the total \( I_{K} \) were different in the two regions. In response to a 200 ms test pulse, a duration corresponding to normal APD in the rabbit ventricles, the amplitude of \( I_{Ki} \) in the apex was about three times greater than that of \( I_{Ki} \). On the contrary, the amplitude of \( I_{Ki} \) at 200 ms test pulse in the base was almost equal to that of \( I_{Ki} \). The activation
Fig. 3. $I_K$ in apical and basal myocytes. $I_K$ was elicited by applying depolarizing potentials to various levels ranging from $-40$ mV to $+60$ mV for 3 s from a holding potential of $-50$ mV. E-4031 (5 μM) was used to separate the two components of $I_K$. In the upper panel, representative current tracings of $I_K$ and its two components (E-4031-sensitive and -insensitive components) are shown for an apical myocyte (A) and a basal myocyte (B) at depolarization to $-40$, $-20$, $0$, $+20$, $+40$, and $+60$ mV, respectively. Horizontal solid lines in the current traces indicate zero current level. The lower panel illustrates the individual data of 18 cells from 7 hearts. The tail current density of $I_K$ and its two components following the depolarization to $+40$ mV was measured in each cell. Each bar represents the data obtained from each myocyte and each cluster of bars represents myocytes from the same heart. The empty bars indicate $I_{Kr}$ and the shaded bars, $I_{Ks}$. The whole bar length represents total tail current density of $I_K$. 
Fig. 4. Voltage-dependent activation of $I_{K}$, $I_{Ks}$, and $I_{Kd}$ in apical and basal myocytes. Protocol was the same as described in Fig. 3. A and B: I±V relationships for the tail current of $I_{K}$, $I_{Ks}$, and $I_{Kd}$. C and D: normalized I±V relationships for the tail current of $I_{K}$, $I_{Ks}$, and $I_{Kd}$. n=7 for apical myocytes (A, C); n=9 for basal myocytes (B, D). In C and D, the tail current amplitudes were normalized to the current amplitude at the most positive potentials, and the relative activation curves were drawn by fitting the averaged data as a function of test potential to Boltzmann distribution ($I/I_{\text{max}} = 1/[1 + \exp(V_{1/2} - V_{t})/k]$), where $V_{1/2}$ is voltage at which half activation was achieved, $V_{t}$ is the test potential, and $k$ is the slope factor.

time constants of $I_{K}$, $I_{Ks}$, and $I_{Kd}$ were not significantly different between the apex and the base (455±58 vs. 425±25 ms for $I_{K}$; 560±48 vs. 464±34 ms for $I_{Ks}$; and 388±66 vs. 309±20 ms for $I_{Kd}$; n=6 for apex and n=8 for base, NS in each group).

3.6. Deactivation kinetics of $I_{K}$

The deactivation time course was examined by two exponential fits of tail currents recorded on repolarization to holding potential of −50 mV after a 3-s pulse to +40 mV. The fast and slow time constants ($\tau_{f}$ and $\tau_{s}$) for the deactivation of each component as well as their relative contributions in apical and basal myocytes were summarized in Table 1. In both apical and basal myocytes, $\tau_{f}$ and $\tau_{s}$ for $I_{K}$ were significantly shorter than those for $I_{Ks}$, whereas no statistical difference was observed between those two regions.
Fig. 5. Time-dependent activation of \( I_K \), \( I_{Ks} \), and \( I_{Kr} \) in apical and basal myocytes. Envelopes of tail current were obtained by applying depolarizing pulses of variable duration (from 100 to 4000 ms) to +40 mV from holding potential of -50 mV. Upper panels of A and B: representative recordings of \( I_K \), \( I_{Ks} \), and \( I_{Kr} \). Horizontal solid lines in the current traces indicate zero current level. Lower panels of A and B: time courses of activation. \( n=6 \) for apical myocytes (A); \( n=8 \) for basal myocytes (B). Time courses of \( I_K \), \( I_{Ks} \), and \( I_{Kr} \) were obtained from a single exponential fit of the initial tail current amplitude as a function of the pulse duration. The activation time constants for \( I_K \), \( I_{Ks} \), and \( I_{Kr} \) in the apical myocytes were 455±58, 560±48 and 388±66 ms, and those in the basal myocytes were 425±58, 464±34 and 309±20 ms, respectively. No significant differences were observed between the time constants of apical and basal myocytes.
Table 1

<table>
<thead>
<tr>
<th>Component</th>
<th>Apex (n = 12)</th>
<th>Base (n = 14)</th>
<th>(\frac{a}{a+b})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I_{Ks})</td>
<td>222 ± 11</td>
<td>212 ± 9</td>
<td>0.62 ± 0.03</td>
</tr>
<tr>
<td>(I_{Ks}+)</td>
<td>1074 ± 41</td>
<td>1022 ± 84</td>
<td>0.56 ± 0.03</td>
</tr>
<tr>
<td>(I_{Ks})</td>
<td>1290 ± 140</td>
<td>1115 ± 89</td>
<td>0.50 ± 0.03</td>
</tr>
<tr>
<td>(I_{Ks}^-)</td>
<td>155 ± 16</td>
<td>187 ± 14</td>
<td>0.67 ± 0.03</td>
</tr>
<tr>
<td>(I_{Ks}^-)</td>
<td>890 ± 83</td>
<td>857 ± 62</td>
<td>0.59 ± 0.04</td>
</tr>
</tbody>
</table>

* The deactivation time course was examined by two exponential fits of tail currents recorded on repolarization to holding potential of \(-50 \text{ mV}\) after a 3-s pulse to \(+40 \text{ mV}\) test potential, according to the equation

\[ Y = a^\tau \exp(-x/\tau_a) + b^\tau \exp(-x/\tau_b) + c \]

where \(a\) and \(b\) are the extrapolated amplitudes of the faster and slower exponential components at the onset of repolarization, and \(c\) is the constant baseline current.

\(^b\) p < 0.05 compared with the corresponding value of \(I_{Ks}^-\).

3.7. Effects of isoproterenol on \(I_{Ks}\) and \(I_{Ks}^-\)

Effects of \(\beta\)-stimulation on \(I_{Ks}\) by isoproterenol (1 \(\mu\text{M}\)) were studied in the presence of E-4031 (5 \(\mu\text{M}\)). The representative recordings are shown in Fig. 6. In the apical myocyte (A) without measurable \(I_{Ks}\) component, isoproterenol induced no appreciable change in either the time-dependent outward current or the tail current. On the contrary, in the basal myocyte (B), which exhibited an obvious \(I_{Ks}\) current, isoproterenol almost doubled the time-dependent outward current and the corresponding tail current at most test potentials. In a total of five basal myocytes, isoproterenol increased the time-dependent outward current by 111 ± 8% during a 3-s depolarizing step at +40 mV and the tail current by 120 ± 9% on repolarization to the holding potential of \(-50 \text{ mV}\). The isoproterenol-induced increase in \(I_{Ks}\) was blocked by the application of 1 \(\mu\text{M}\) propranolol (data not shown).

Effect of isoproterenol on \(I_{Ks}\) were examined in apical myocytes after blocking \(I_{Ks}^-\) by the application of 30 \(\mu\text{M}\) chromanol 293B. The representative recordings are shown in Fig. 7. Isoproterenol (1 \(\mu\text{M}\)) induced no significant change of the time-dependent outward current as well as the tail current, indicating that it did not affect \(I_{Ks}^-\). Similar results were observed in three apical and two basal myocytes.

These results indicate that the E-4031-insensitive current (\(I_{Ks}^-\)) is largely enhanced by \(\beta\)-adrenergic stimulation.

![Fig. 6. Augmentation of \(I_{Ks}\) by isoproterenol. The voltage protocol was the same as that in Fig. 3. Currents were recorded before and after application of E-4031 (5 \(\mu\text{M}\)) and then after addition of isoproterenol (1 \(\mu\text{M}\)). Horizontal solid lines in the current traces indicate zero current level. In the apical myocyte (A) without a measurable \(I_{Ks}\) component, isoproterenol induced no appreciable change of the remaining current. In the basal myocyte (B), which demonstrated an obvious \(I_{Ks}^-\), isoproterenol almost doubled the time-dependent outward current during the 3-s depolarizing step and the tail current on repolarization to the holding potential of \(-50 \text{ mV}\).](image-url)
region of Langendorff-perfused rabbit ventricles (61% vs. 38% increase in the Q–T interval) [24]. In the present study, we demonstrated a regional difference along the apex/base axis of rabbit left ventricle; \( I_{\text{Kr}} \) was more prominently expressed in the apex than base, whereas \( I_{\text{Ks}} \) expression was more abundant in the base than in the apex, giving rise to a large difference in the \( I_{\text{Kr}}/I_{\text{Ks}} \) ratio.

Many lines of evidences suggest that prominent electrophysiological heterogeneity, especially the transmural heterogeneity between the epicardial and endocardial regions, exists within the mammalian ventricle, largely because of diversity and varying density of repolarizing K⁺ currents. Furukawa et al. reported that \( I_{\text{Ko}} \) density was larger in epicardial than endocardial myocytes (4.21±0.83 vs. 2.86±0.73 pA/μF) of feline ventricle, and that its activation kinetics were also different in the two layers [29]. In canine ventricle, \( I_{\text{Kr}} \) tail current density was similar among the epicardial, endocardial and M cells, whereas \( I_{\text{Ks}} \) tail current density was significantly smaller in the M cells (0.92±0.14 pA/μF) than in epi- and endocardial cells (1.99±0.30 and 1.83±0.18 pA/μF, respectively), resulting in a much longer APD in M cells [7]. As has been reported in other species, epi- to endocardial differences in the repolarizing K⁺ currents may also exist in rabbit ventricles. A wide cell-to-cell variation in the amplitude of \( I_{\text{Kr}} \) and \( I_{\text{Ks}} \) within the apex or base in the present study could imply epi- to endocardial differences of these currents. Further study is needed to clarify this point.

Recently, Brahmajothi et al. reported a regional heterogeneity of ERG transcript and protein expression in ferret hearts (the epicardial layers of the ventricles), indicating that the expression was more abundant in the apex than in the base [30]. This is consistent with our finding that \( I_{\text{Ko}} \) component was poorly expressed in basal myocytes.

The relative contributions of \( I_{\text{Kr}} \) and \( I_{\text{Ks}} \) in ventricular myocytes are species dependent. In canine ventricular myocytes, \( I_{\text{Kr}} \) density is approximately five-fold greater than that of \( I_{\text{Ks}} \), as measured from tail currents at \(-35 \text{ mV} \) after 3-s test pulses to \(+65 \text{ mV} \) [31]. The ratio of \( I_{\text{Kr}}/I_{\text{Ks}} \) is 11.4 in guinea pig myocytes, as measured at \(-40 \text{ mV} \) after 7.5 s test pulses to \(+60 \text{ mV} \) [25]. In rabbit ventricular myocytes, we firstly demonstrated a regional difference in the relative contribution of \( I_{\text{Ko}} \) and \( I_{\text{Kr}} \); \( I_{\text{Ko}} \) amplitude was about three-fold of \( I_{\text{Kr}} \) in basal myocytes, but only about half of \( I_{\text{Ko}} \) in apical myocytes, as measured from the tail currents at \(-50 \text{ mV} \) after 3-s test pulses to \(+40 \text{ mV} \). In the whole left ventricle of rabbit, Salata et al. reported a \( I_{\text{Kr}}/I_{\text{Ks}} \) ratio of 13.9 based on the experiments using voltage ramps, where \( I_{\text{Kr}} \) was measured as the maximum doxifiltide-sensitive current at \(-10 \text{ mV} \) and \( I_{\text{Ks}} \) as the amplitude of outward current at \(+60 \text{ mV} \) relative to holding current [23]. This value is much larger than the ratio obtained in the present study. The reason for this difference is unknown. As for human ventricular myocytes, the ratio is about 1.6, when calculated from the

4. Discussion

4.1. Regional differences in \( I_{\text{K}} \) components

We have proposed that \( I_{\text{Kr}} \) is expressed at higher density in the apical than basal region of rabbit ventricles, since we observed experimentally that E-4031 (0.1 μM) prolonged repolarization more significantly in the apical than basal

whereas chromanol 293B-insensitive current (\( I_{\text{Ko}} \)) is resistant to the β-stimulation.

Fig. 7. Lack of effects on \( I_{\text{Kr}} \) by isoproterenol. \( I_{\text{Kr}} \) was elicited in an apical myocyte by applying depolarizing potentials to various levels ranging from \(-40 \text{ mV} \) to \(+10 \text{ mV} \) for 3 s from a holding potential of \(-50 \text{ mV} \). Currents were recorded before and after application of chromanol 293B (30 μM) and then after addition of 1 μM isoproterenol in an apical myocyte. Isoproterenol induced no appreciable change of the time-dependent outward current during the 3-s depolarizing step and the tail current on repolarization to the holding potential of \(-50 \text{ mV} \). Additional application of 5 μM E-4031 resulted in complete elimination of both the time-dependent outward current and the tail current. The remaining time-independent current during depolarization may reflect chloride current enhanced by isoproterenol.

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data of tail current density obtained at $-35$ mV after a 3-s test pulse to $+40$ mV [18]. This value is comparable to the ratio observed in our experiments on rabbit ventricular myocytes. Such differences in the ratio of $I_K/|I_K|$ may be important in understanding the effects of class III agents on the ventricular repolarization of different animal species.

4.2. Characteristics of rabbit $I_K$, and $I_{Kr}$

Our data on the kinetics of the E-4031-sensitive and-insensitive components ($I_{Kr}$ and $I_{Ks}$) are inconsistent with those previously described by Sanguinetti and Jurkiewicz in guinea pig ventricular myocytes [25] in the following aspects. First, rabbit $I_{Kr}$ observed in our experiments had a less negative half-activation voltage ($V_{1/2}$: $-8.7 \pm 2.2$ mV in apex and $-12.9 \pm 1.6$ mV in base), than guinea pig $I_{Kr}$ ($V_{1/2}$: $-21.5$ mV) [25], and the former reached saturation at $+0$ mV, showing less prominent inward rectification. Second, rabbit $I_{Ks}$ had a more negative half-activation voltage ($-2.2 \pm 1.5$ mV in apex and $-3.3 \pm 1.9$ mV in base) than guinea pig $I_{Ks}$ ($V_{1/2}$: $+15.7$ mV) [25]. Third, the activation time course of rabbit $I_{Kr}$ (time constant: $388 \pm 66$ ms in apex and $309 \pm 20$ ms in base) was about ten times slower than that (within $30$ ms at $+30$ mV) of guinea pig $I_{Kr}$ [25]. Finally, the time course of activation of rabbit $I_{Ks}$, was about three times faster than that of guinea pig $I_{Ks}$; rabbit $I_{Ks}$ appears to be saturated at about 2–3 s, whereas guinea pig $I_{Ks}$ needs 7.5 s before saturation. Accordingly, it is difficult in rabbit ventricular myocytes, unlike in guinea pig, to discriminate $I_{Kr}$ and $I_{Ks}$ based on their activation kinetics.

The above-mentioned differences in activation kinetics of $I_K$ components may reflect species differences of the currents. The activation time constant ($352.7 \pm 9.45$ ms) of $I_{Kr}$ (dofetilide-sensitive current) measured by Carmeliet [15] in rabbit ventricular myocytes is quite similar to ours described above. Furthermore, Beatch et al. reported that rabbit $I_{Ks}$ (sematilide-insensitive component) reached saturation $\sim 2$ s at $+10$ mV depolarizing pulse [22].

The composition of external solution might affect the two components of $I_K$. For example, a solution containing Na$^+$ but neither K$^+$ nor Ca$^{2+}$ diminished $I_{Kr}$ (by about 50% at $-10$ mV) but increased $I_{Ks}$, by about 30% at $+50$ mV) [28]. However, as far as rabbit ventricular myocytes are concerned, we confirmed that the tail current of $I_{Kr}$ (after blocking the $I_{Kr}$ component by 30 $\mu$M chromanol 293B) was almost unaffected by the change of the external Tyrode’s solution to the K$^+$- and Na$^+$-free but Ca$^{2+}$-containing NMG solution. This observation is consistent with the results obtained in rabbit cardiac Purkinje cells by Scamps and Carmeliet [32]. They demonstrated that a reduction of extracellular K$^+$ from 5.4 mM to 0.2 mM in the absence of extracellular Na$^+$ did not decrease $I_K$, which showed a $I_{Kr}$-like voltage-dependence. These results indicate that the modulation of $I_{Kr}$ by the extracellular K$^+$-free condition is complicated by the simultaneous change of other ionic compositions. Further experiments are needed to clarify this issue.

4.3. Regional difference in APD and its underlying mechanisms

Our study demonstrated that at 1 Hz stimulation APD was significantly longer in apical than in basal myocytes, and that APD difference between the apex and base was enhanced after the application of E-4031. As shown in Fig. 5, after a 200-ms pulse to $+40$ mV, which mimics the action potential configuration of ventricular muscle, $I_K$ tail was significantly smaller in apical than basal myocytes. This may explain in part why the APD was longer in the apex than in the base. Moreover, the tail current amplitude of $I_K$ was about three-fold as large as $I_{Kr}$ in the apex, but it was approximately equal to $I_{Ks}$ in the base. Therefore, the relative contribution of $I_{Kr}$ to repolarization may be much greater in the apical than the basal myocytes. These results may explain why E-4031 induces a more significant prolongation of APD in apical than basal myocytes of the rabbit ventricles.

The present study suggests that differences in the density of $I_K$ components may contribute mainly to the regional differences in ventricular repolarization. However, these data do not exclude roles of other K$^+$ currents such as the transient outward current ($I_o$). In fact, a greater amplitude of $I_o$ in the epicardial than endocardial myocytes has been demonstrated in canine, feline, rat, rabbit and human ventricles [6,33–37]. However, since $I_o$ in rabbit ventricular myocytes has a very slow recovery from inactivation (a time constant longer than 1 sec) [12,38,39], it mainly contributes to repolarization at stimulating frequencies lower than 1 Hz, and its role may be less important than that of $I_K$ at the physiological heart rates.

4.4. Potential limitations

In our study, the inward rectification of the time-dependent outward current of $I_{Kr}$ was relatively weak and showed large cell-to-cell variation. Since the data of $I_{Kr}$ were obtained by digital subtraction of the E-4031-insensitive current from the control current, it may contain other contaminating currents in addition to $I_{Kr}$. In addition, use of extracellular K$^+$-free solution modified the rectification properties of $I_{Kr}$. Therefore, our conclusion on the regional difference of $I_K$ and its two components is drawn mainly from the data of the tail current. Furthermore, a wide cell-to-cell variation in the density of $I_{Kr}$ and $I_{Ks}$ within the apex or base in the present study may imply epicardial difference of these currents. Further study is needed to clarify this point.
4.5. Physiological and clinical significance

In summary, the present study demonstrated the regional differences in $I_{Ks}$ and its components ($I_{Kr}$ and $I_{Ks}$) between the apical and basal regions of rabbit ventricles. These differences may contribute to the regional disparity in APD and the sensitivity to prolongation by methanesulfonanilide class III antiarrhythmic agents. These findings have important implications in understanding the cardiac electrophysiology as well as the proarrhythmic mechanisms of class III agents in rabbit hearts. Heterogeneity in action potential duration and refractoriness has been reported to favor the initiation of re-entrant arrhythmias [40,41]. Thus, the greater APD prolongation by methanesulfonanilide class III antiarrhythmic agents such as E-4031 in the apex than the base may lead to a greater spatial inhomogeneity of ventricular repolarization (especially at decreased heart rates), initiating reentrant tachyarrhythmias.

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