Density and Kinetics of $I_{\text{Kr}}$ and $I_{\text{Ks}}$ in Guinea Pig and Rabbit Ventricular Myocytes Explain Different Efficacy of $I_{\text{Ks}}$ Blockade at High Heart Rate in Guinea Pig and Rabbit

Implications for Arrhythmogenesis in Humans

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Background—Class III antiarrhythmic agents commonly exhibit reverse frequency-dependent prolongation of the action potential duration (APD). This is undesirable because of the danger of bradycardia-related arrhythmias and the limited protection against ventricular tachyarrhythmias. The effects of blockade of separate components of delayed rectifier K$^+$ current ($I_k$) may help to develop agents effective at high heart rate.

Methods and Results—We assessed the density and kinetics of the 2 components of the delayed rectifier K$^+$ current, $I_{\text{Kr}}$ and $I_{\text{Ks}}$, in rabbit and guinea pig ventricular myocytes. The effects of their specific blockers (chromanol 293B for $I_{\text{Kr}}$ and E-4031 for $I_{\text{Ks}}$) on the action potential was studied at different heart rates by use of whole-cell patch-clamp techniques. In guinea pig ventricular myocytes only, blockade of $I_{\text{Ks}}$ causes APD prolongation in a frequency-independent manner, whereas blockade of $I_{\text{Kr}}$ in rabbit ventricular myocytes shows reverse frequency dependence, as does blockade of $I_{\text{Ks}}$ in both species. This result can be explained primarily by the higher density of $I_{\text{Kr}}$ in guinea pig ventricle and by its slow deactivation kinetics, which allows $I_{\text{Kr}}$ to accumulate at high heart rate because little time is available for complete deactivation of it during diastole.

Conclusions—Density and kinetics of components of $I_k$ explain why blockade of $I_{\text{Ks}}$ is more effective at high heart rate in the guinea pig ventricle than in the rabbit ventricle, without adverse effects at low heart rate. (Circulation. 2001;104: 951-956.)

Key Words: potassium ion channels antiarrhythmia agents action potentials

The delayed rectifier potassium current ($I_k$), a major determinant of action potential duration (APD), has a rapidly ($I_{\text{Kr}}$) and a slowly ($I_{\text{Ks}}$) activating component. They differ in kinetic properties, rectification characteristics, and sensitivity to drugs. Most of the class III antiarrhythmic drugs, such as dofetilide and E-4031, prolong APD in a reverse frequency-dependent manner by blockade of $I_{\text{Kr}}$. Therefore, these $I_{\text{Kr}}$ blockers may act in a proarrhythmic manner during bradycardia, with minimal therapeutic potency against tachyarrhythmias. Chromanol 293B has recently been reported to selectively block $I_{\text{Kr}}$. Moreover, it prolonged APD in a frequency-independent manner in guinea pig and human ventricular myocytes. This favorable characteristic of chromanol 293B may potentially have an antiarrhythmic effect on ventricular tachyarrhythmias without the harmful effect at low heart rate.

$I_{\text{Ks}}$ has been reported to be unevenly distributed over the ventricles. It is larger in epicardium and endocardium than in midmyocardium, in the right than in the left canine ventricle, and at the base than the apex in the rabbit ventricles. The removal of these 3 types of regional inhomogeneities by pharmacological blockade may render the heart electrically more homogeneous.

From the temporal point of view, $I_{\text{Kr}}$ and $I_{\text{Ks}}$ display rather different activation and deactivation kinetics in ventricular myocardium of rat, guinea pig, rabbit, and dog. Compared with guinea pigs, $I_{\text{Kr}}$ in rabbit ventricular myocardium activates ~10 times more slowly, although $I_{\text{Ks}}$ activates ~3 times faster. Such fundamental differences in channel kinetics may be expected to have a bearing on APD prolongation and on the efficacy of different class III antiarrhythmic agents.

We compared the densities and the kinetics of $I_{\text{Kr}}$ and $I_{\text{Ks}}$ in guinea pig and rabbit ventricular myocytes and assessed the effects of E-4031 ($I_{\text{Kr}}$ blocker) and chromanol 293B ($I_{\text{Ks}}$ blocker) on APD. Specific action potential prolongation at short cycle length is feasible by $I_{\text{Kr}}$ blockade in the guinea pig, but not in the rabbit. This difference is consistent with (1) the higher density of $I_{\text{Kr}}$ in the guinea pig and (2) its slow deactivation, which allows little time for decrease of the...
current during diastole. Because $I_{Ks}$ blocker produces APD prolongation at short cycle length in humans as in guinea pig, it is suggested that $I_{Ks}$ is relevant in human ventricle.

### Methods

#### Isolation of Ventricular Myocytes

Japanese White rabbits (1.5 to 2.0 kg) or guinea pigs (200 to 300 g) were euthanized under anesthesia with thiamylal sodium or pentobarbital sodium after being heparinized. Single myocytes were isolated enzymatically from the middle of the left ventricular free wall by a procedure described previously. All animal procedures were approved by the Animal Care and Use Committee, Research Institute of Environmental Medicine, Nagoya University.

#### Electrophysiological Recordings

A single-pipette whole-cell patch-clamp method was used to record the action potential and current. The resistance of the glass pipette was 4 to 6 MΩ after it was filled with an internal pipette solution. The cell capacitance was determined by applying a ramp voltage pulse of $\approx 0.5$ V/s at a potential ranging between $-50$ and $+70$ mV. The cell capacitance and series resistance were electrically compensated by $\approx 70\%$. Action potentials were recorded in Tyrode’s solution and were elicited by application of a 5-ms depolarizing pulse through the pipette and recorded at cycle length from 333 to 10,000 ms. The APD was measured at 90% repolarization (APD$_{90}$).

#### Solutions and Drugs

Tyrode’s solution, used for cell isolation and the recording of action potentials, was composed of (in mmol/L) NaCl 143, KCl 5.4, MgCl$_2$ 0.5, NaH$_2$PO$_4$ 0.25, HEPES 5.0, CaCl$_2$ 1.8, and glucose 5.6 (pH 7.35 adjusted with NaOH). The internal pipette solution was composed of (in mmol/L) KOH 60, KCl 80, aspartate 40, HEPES 5.0, EGTA 10, MgATP 5.0, sodium creatinine phosphate 5.0, and CaCl$_2$ 0.65 (pH 7.2 adjusted with NaOH; pCa 8.0). When $I_{Ks}$ was measured, cells were superfused with a Na$^+$- and K$^+$-free solution (NMG solution) composed of (in mmol/L) N-methyl-D-glucamine 149, MgCl$_2$ 5, CaCl$_2$ 0.9, HEPES 5.0, and nisoldipine 0.003 (pH 7.35 adjusted with HCl). The bath temperature in all experiments was 35°C to 37°C.

$I_{Ks}$ was measured during blockade of $I_{Kr}$ by 10 μmol/L E-4031 added to the superfusate, and $I_{Kr}$ was measured during blockade of $I_{Ks}$ by 30 μmol/L chromanol 293B. Action potentials were measured before and after perfusion of 10 μmol/L of each drug for 10 minutes. E-4031 was dissolved in distilled water. Chromanol 293B was dissolved in dimethyl sulfoxide (DMSO) as 100 mmol/L stock solutions and diluted in superfusates to achieve a final concentration immediately before each application. The final concentrations of DMSO (0.01% to 0.03%) had no significant intrinsic effects on the current traces and action potential configuration.

### Statistical Analysis

Data were expressed as mean±SEM. Results were compared by Student’s $t$ test for paired and unpaired data to evaluate statistical significance, and differences were considered significant at $P<0.05$. In analysis of activation and deactivation kinetics of $I_{Ks}$ and $I_{Kr}$, the double-exponential fit was accepted as the fit of choice whenever it had a mean square error that was at least one third that obtained with a single exponential.

### Results

#### Effects of 293B and E-4031 on Action Potentials

Action potentials were measured in the presence and absence of 10 μmol/L of the 2 agents in guinea pig and rabbit ventricular myocytes at the shortest cycle length (333 ms) and at a longer cycle length (1000 ms) (Figure 1). Obviously, both blockers prolonged the action potential in both species and at both cycle lengths. Blockade of $I_{Ks}$ caused a larger increase in APD in the guinea pig at 333 ms than at 1000 ms and the opposite in the rabbit. Blockade of $I_{Kr}$ caused virtually no increase in APD in the rabbit at 333 ms. In both species, the increase in APD during blockade of $I_{Ks}$ was larger at 1000 ms than at 333 ms.

Figure 2 shows cycle length (333 ms to 10 seconds) versus APD before and after administration of 10 μmol/L of the blocker in both species. As in Figure 1, reverse frequency dependence is obvious for $I_{Ks}$ blockade in both species and for $I_{Kr}$ blockade in the rabbit, but not the guinea pig. It may further be appreciated that APD shortens at excessively long cycle lengths in the rabbit, a well-known phenomenon due to the slow recovery from inactivation of rabbit transient outward current ($I_{Kr}$). Figure 3 addresses the issue of reverse frequency dependence in more detail by comparison of the increase in APD in the 2 species under the influence of both blockers and at 3 selected cycle lengths. Obviously, only $I_{Ks}$ block in the guinea pig fulfills the criterion of substantial increase in APD without excessive increase at long cycle length.

### Density of $I_{Ks}$ and $I_{Kr}$

Figure 4A shows the representative total $I_K$ and the separated $I_{Ks}$ and $I_{Kr}$, as the E-4031–resistant and chromanol 293B–resistant currents, respectively, elicited from a holding potential of $-50$ mV to a step potential of 3 seconds’ duration from $-40$ mV to $+50$ mV at 0.1 Hz. Figure 4B shows the current-voltage relationship of the time-dependent outward (step) current at the end of the step potential (top) and the tail

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**Figure 1.** Rate dependence of APD prolongation by chromanol 293B (10 μmol/L) and E-4031 (10 μmol/L) in guinea pig and rabbit ventricular myocytes. Representative action potentials were recorded at cycle length of 333 and 1000 ms under control conditions, in presence of 293B, and in presence of E-4031.
currents after stepping back to the holding potential (bottom) for \( I_{Ks} \) and \( I_{Kr} \). Inward rectification of \( I_{Kr} \) is obvious in both species. The total \( I_K \) current is substantially larger in the guinea pig than in the rabbit, because \( I_{Ks} \) is larger in guinea pig than in rabbit. In fact, at the relevant potential range of +20 to +30 mV, which occurs during repolarization, \( I_{Ks} \) in guinea pig is still larger than \( I_{Kr} \) and \( I_{Ks} \) together in rabbit.

**Activation and Deactivation Kinetics of \( I_{Ks} \) and \( I_{Kr} \)**

Figure 5 shows the voltage-dependent activation properties of total \( I_K \) and \( I_{Ks} \) and \( I_{Kr} \) tails of guinea pig and rabbit ventricular myocytes. Figure 5B shows that the voltage at which half activation is achieved (\( V_{th} \)) for \( I_{Kr} \) is similar in rabbit and in guinea pig (\(-21.9 \pm 1.4 \) and \(-20.6 \pm 2.5 \) mV, respectively). Figure 5C, however, shows a substantially more negative \( V_{th} \) for \( I_{Ks} \) in rabbit (\(-1.2 \pm 1.7 \) mV) than in guinea pig (\(+18.2 \pm 1.7 \) mV). Consequently, total \( I_K \) was activated at more negative potential in rabbit than in guinea pig (Figure 5A: rabbit \( V_{th} \) = \(-8.6 \pm 1.1 \) mV; guinea pig \( V_{th} \) = +8.1 \pm 3.5 mV).

Figure 6 illustrates the results of envelope-of-tails tests performed in guinea pig and rabbit ventricular myocytes. Envelopes of tail currents were evoked by applying depolarizing pulses to +50 mV from a holding potential of −50 mV, with duration ranging from 100 to 1900 ms for \( I_{Ks} \) (in the presence of E-4031) and from 25 to 2100 ms for \( I_{Kr} \) (in the presence of chromanol 293B). Tail currents after each pulse were measured on return to −50 mV. Figure 6A shows representative tracings of \( I_{Ks} \) and \( I_{Kr} \) in guinea pig and rabbit ventricular myocytes. Figure 6B shows the averaged time courses of tail envelopes obtained by fitting the tail current

**Figure 3.** APD prolongations as a function of cycle length (333, 1000, and 10 000 ms) in guinea pig and rabbit ventricular myocytes. Data are mean ± SEM. \( n = 12 \) and 10 in 10 \( \mu \)mol/L 293B– and 10 \( \mu \)mol/L E-4031–treated guinea pig ventricular myocytes; \( n = 10 \) and 8 in 10 \( \mu \)mol/L 293B– and 10 \( \mu \)mol/L E-4031–treated rabbit ventricular myocytes, respectively.

**Figure 4.** \( I_{Ks} \), \( I_{Kr} \), and \( I_{Kr} \) in guinea pig and rabbit ventricular myocytes. Currents were elicited by applying depolarizing potentials to various levels ranging from −40 to +50 mV for 3 seconds from a holding potential of −50 mV. \( I_{Ks} \) and \( I_{Kr} \) were obtained as E-4031 (10 \( \mu \)mol/L)–resistant current and chromanol 293B (30 \( \mu \)mol/L)–resistant current. A, Representative current traces for guinea pig and rabbit ventricular myocytes. B, I-V relationships for step current and tail current of \( I_{Ks} \) and \( I_{Kr} \) in guinea pig (left) and rabbit (right) ventricular myocytes. \( n = 10 \) for guinea pig and \( n = 12 \) for rabbit.

**Figure 2.** Quantitative data of rate dependence of APD in guinea pig and rabbit ventricular myocytes under control conditions, in presence of 10 \( \mu \)mol/L 293B, and in presence of 10 \( \mu \)mol/L E-4031. Data are mean ± SEM. *P < 0.05 for difference between drug effect and control value at each frequency. \( n = 12 \) and 10 in 293B– and E-4031–treated guinea pig ventricular myocytes; \( n = 10 \) and 8 in 293B– and E-4031–treated rabbit ventricular myocytes, respectively.
amplitude to a single exponential function of the pulse duration with numerical data in the Table.

The deactivation time constants were examined by double-exponential fit of tail currents recorded on repolarization to a potential of −50 mV after a 3-second pulse to +50 mV. The Table summarizes the fast and slow time constants (τf and τs) for the deactivation of each component in guinea pig and rabbit ventricular myocytes. Average values of both deactivation time constants (τf and τs) of guinea pig Ik were longer

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<th>Time Constants of the Activation and Deactivation Kinetics of Ik and Ir in Guinea Pig and Rabbit Ventricular Myocytes</th>
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Values are mean±SEM. n indicates number of cells. The activation time constants at +50 mV of Ik were approximated by a single-exponential function. The activation time constants of Ir at +50 mV were approximated by a single-exponential function in guinea pig and by a double-exponential function in rabbit. The deactivation time constants were approximated by a double-exponential fit of tail currents recorded at −50 mV after a 3-second pulse to +50 mV from a holding potential of −50 mV.

*P<0.05; NS, no significant difference.
ventricular tissue preparations of guinea pig,21 rabbit,22 and dog.23 Different intracellular milieu might be involved in the discrepancy.

E-4031 and dofetilide are recently developed, pure class III drugs. They selectively inhibit $I_{Kr}$, as did E-4031 in this study, and show reverse frequency dependence in the prolongation of APD in canine,12 guinea pig,3 and rabbit ventricular myocytes.13,20 Use dependence has been reported for the effect of E-4031 on $I_{Kr}$ in rabbit ventricular myocytes, although reverse frequency dependence has been demonstrated for its effects on APD.24

Relevance for the Human Ventricle
In human ventricular myocytes, the presence of $I_{Kr}$ is still a debated issue.14,25–27 The evidence for the relevance of its blockade by chromanol 293B is indirect and based on the similarity of action potential prolongation in guinea pig and human ventricular myocytes at high heart rate.6 In right ventricular myocytes isolated from explanted human hearts with primarily left heart failure, Li et al14 demonstrated the presence of $I_{Kr}$, with relatively slow activation kinetics ($\tau_{a}$ of 360 ms and $\tau_{d}$ of 8.5 seconds at $+50$ mV). Recently, Virág et al25 showed $I_{Kr}$ in undiseased human left ventricular myocytes with slow activation ($\tau$ of 903 ms at $+50$ mV) and relatively rapid deactivation ($\tau$ of 122 ms at $-40$ mV). Recent developments in the research field of the congenital long-QT (LQT) syndrome indicate that dysfunction of both $I_{Kr}$ and $I_{Ks}$ may be the cause of some forms of the LQT syndrome. One (LQT2) results from mutations in the HERG gene, and another (LQT1) results from mutations in the KVLQT1 gene. These studies strongly suggest roles for $I_{Kr}$ and $I_{Ks}$ in the repolarization of the human ventricular action potential.28 It is essential to determine the kinetic properties of $I_{Kr}$ and $I_{Ks}$ as well as their relative densities in human ventricle to understand the repolarization process and the mechanism underlying tachyarrhythmias.

Limitations
E-4031 at 10 $\mu$mol/L completely inhibited $I_{Kr}$. The concentrations of chromanol 293B (10 and 30 $\mu$mol/L) were comparable to that used by Bosch et al.6 Chromanol 293B blocked $I_{Kr}$ by 70% at 10 $\mu$mol/L and by 100% at 30 $\mu$mol/L, at which concentration it also blocks $>50\%$ of $I_{Kr}$. Therefore, we used 10 $\mu$mol/L chromanol 293B to assess the effects of $I_{Kr}$ blockade on APD. It can thus not be ruled out that the effect of blockade of $I_{Kr}$ on APD prolongation was underestimated. We used 30 $\mu$mol/L chromanol 293B to block $I_{Kr}$ completely during the assessment of $I_{Kr}$. After application of 30 $\mu$mol/L 293B, a current with obvious rectifying properties was left. An additional 10 $\mu$mol/L E-4031 blocked this current completely (data not shown). This implies that $I_{Kr}$ can be defined as chromanol 293B–resistant current. The activation and deactivation time constants of rabbit $I_{Kr}$ in the present study are shorter than those we reported previously.9 This could be due to slightly different experimental conditions in bath temperature and E-4031 concentrations.

It should be emphasized that the mechanism responsible for the frequency dependence of APD prolongation is not caused only by $I_{Kr}$ and $I_{Ks}$. Other currents, such as the inward
rectifier current \( (I_{K1}) \), \( Ca^{2+} \) inward current, \( Na^{+}-K^{+} \) pump current, \( Na^{+}-Ca^{2+} \) exchanger current, and slowly inactivating \( Na^{+} \) current, also contribute to frequency dependence of APD.\(^{29,30}\) In addition, the contribution of these currents may be different in atrium and ventricle. Further experimental studies, especially in human tissues, are of prime importance to elucidate the issue.

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