Transmembrane $I_{\text{Ca}}$ contributes to rate-dependent changes of action potentials in human ventricular myocytes

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Departments of 1Medicine and 2Surgery, Montreal Heart Institute and University of Montreal, and 3Department of Pharmacology, McGill University, Montreal, Quebec, Canada H1T 1C8

Li, Gui-Rong, Baofeng Yang, Jianlin Feng, Ralph F. Bosch, Michel Carrier, and Stanley Nattel. Transmembrane $I_{\text{Ca}}$ contributes to rate-dependent changes of action potentials in human ventricular myocytes. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H98–H106, 1999.—The mechanism of action potential abbreviation caused by increasing rate in human ventricular myocytes is unknown. The present study was designed to determine the potential role of $\text{Ca}^{2+}$ current ($I_{\text{Ca}}$) in the rate-dependent changes in action potential duration (APD) in human ventricular cells. Myocytes isolated from the right ventricle of explanted human hearts were studied at 36°C with whole cell voltage and current-clamp techniques. APD at 90% repolarization decreased by 36 ± 4% when frequency increased from 0.5 to 2 Hz. Equimolar substitution of Mg$^{2+}$ for $\text{Ca}^{2+}$ significantly decreased rate-dependent changes in APD (to 6 ± 3%, $P < 0.01$). Peak $I_{\text{Ca}}$ was decreased by 34 ± 3% from 0.5 to 2 Hz ($P < 0.01$), and $I_{\text{Ca}}$ had recovery time constants of 65 ± 12 and 683 ± 39 ms at −80 mV. Action potential clamp demonstrated a decreasing contribution of $I_{\text{Ca}}$ during the action potential as rate increased. The rate-dependent slow component of the delayed rectifier $K^+$ current ($I_{\text{Ks}}$) was not observed in four cells with an increase in frequency from 0.5 to 3.3 Hz, perhaps because the $I_{\text{Ks}}$ is so small that the increase at a high rate could not be seen. These results suggest that reduction of $\text{Ca}^{2+}$ influx during the action potential accounts for most of the rate-dependent abbreviation of human ventricular APD.

Calcium current; ion channels; action potential; electrophysiology; cardiac arrhythmias

IT IS WELL KNOWN that the cardiac action potential duration (APD) changes with alterations in frequency and APD is significantly abbreviated at rapid rates (3, 10, 15, 35), but the underlying mechanisms are unclear. In mammalian cardiac tissue, rate-dependent abbreviation of APD at rapid rates has been considered to result from an increase in transmembrane $K^+$ conductance (22) and/or an augmentation of $\text{Na}^{+}$-$\text{K}^+$ pump activity (11).

Beeler and Reuter (6) reported that membrane $\text{Ca}^{2+}$ current ($I_{\text{Ca}}$) is important for determining the plateau phase of the action potential in guinea pig ventricular myocardial fibers. Hume and Uehara (19) found that amplitude and kinetics of $I_{\text{Ca}}$ are major determinants of the differences in morphology and duration of the action potential in guinea pig atrial and ventricular myocytes. We also found that $I_{\text{Ca}}$ plays an important role in determining APD in human atrium (25). Therefore, $I_{\text{Ca}}$ changes may explain rate-dependent changes in cardiac APD. However, whether $I_{\text{Ca}}$ contributes to the rate-dependent abbreviation of APD in human ventricle is unknown.

The properties of $I_{\text{Ca}}$ have been described in cardiac cells isolated from human atria and human ventricles (14, 26, 28, 29). However, $I_{\text{Ca}}$ was characterized at room temperature in most of these studies. It has been demonstrated that changes in temperature profoundly affect the availability and/or time-dependent properties of $I_{\text{Ca}}$ (1). Therefore, it is important to evaluate the role of $I_{\text{Ca}}$ in governing rate-dependent changes in APD in humans at normal physiological temperature.

The present study was designed to determine the relation between rate-dependent changes in APD and changes in $I_{\text{Ca}}$ in human ventricular myocytes at a physiological temperature (36°C). The action potential-voltage clamp ("action potential clamp") technique was used to analyze the potential contribution of changes in $I_{\text{Ca}}$ to frequency-dependent changes in APD.

METHODS

Myocyte isolation. Right ventricular tissues from explanted hearts were obtained at the time of heart transplantation from patients. All hearts were initially placed in cold (4°C) oxygenated Krebs solution and then transferred to cardioplegic solution for dissection and coronary artery cannulation. A procedure described previously (24) was used to isolate ventricular cells. Briefly, a portion of the free wall of the right ventricle (~2 × 4–5 cm) was removed along with the coronary artery branch irrigating it, with dissection and arterial cannulation completed within 20 min of excision of the heart. The free wall was perfused with oxygenated, nominally $\text{Ca}^{2+}$-free Tyrode solution for 20–30 min, and the solution was then changed to one containing 200–300 U/ml collagenase (CLS II, Worthington Biochemical, Freehold, NJ) for 60–100 min. Myocytes were isolated from the digested tissue and placed in a high-$K^+$ storage solution (24).

Cells were obtained from five hearts. The underlying heart disease was congestive cardiomyopathy in three cases and left heart failure due to aortic valve disease in two cases. Examination of the right ventricle by a cardiologist pathologist revealed it to be microscopically normal in two cases, to show mild subendocardial fibrosis in one case, and to show cellular hypertrophy in two cases.

A small aliquot of the solution containing the isolated cells was placed in an open perfusion chamber (1 ml) mounted on the stage of an inverted microscope. Myocytes were allowed to adhere to the bottom of the dish for 5–10 min and then superfused at 2–3 ml/min with Tyrode solution. Experiments were conducted at 36°C with temperature controlled by a Peltier-effect device. Only quiescent rod-shaped cells showing clear cross striations were used.
Solutions and chemicals. Tyrode solution contained (mM) 136 NaCl, 5.4 KCl, 1.0 MgCl_2, 2.0 CaCl_2, 0.33 NaH_2PO_4, 10.0 glucose, and 10 HEPES; pH was adjusted to 7.4 with NaOH. For cell dissociation, Ca^{2+} was omitted. A choline solution containing (mM) 136 choline chloride, 5.4 CsCl, 1.0 MgCl_2, 2.0 CaCl_2, 0.33 NaH_2PO_4, 10 glucose, and 10 HEPES was used when ICa was measured directly; pH was adjusted to 7.4 with CsOH. The pipette solution for ICa, recording (whole cell voltage-clamp mode) contained (mM) 20 CsCl, 110 cesium aspartate, 1.0 MgCl_2, 10 HEPES, 0.05 EGTA [5 for the slow component of the delayed rectifier K+ current (IK1), recording), 0.1 GTP, 5 MgATP, and 5 sodium phosphocreatine; pH was adjusted to 7.2 with CsOH.

Electrophysiology and data analysis. The tight-seal whole cell patch-clamp technique was used. Borosilicate glass electrodes (1.0 mm OD) were pulled with a Brown-Flaming puller (model P-87); tip resistances were 2–3 MΩ when filled with pipette solution. Data were acquired with the use of an Axopatch 200A and/or 1-D amplifier (Axon Instruments, Foster City, CA). Command pulses were generated by a 12-bit digital-to-analog converter controlled by pCLAMP software (Axon Instruments). Recordings were low-pass filtered at 2 kHz and stored on the hard disk of an IBM-compatible computer. Tip potentials were compensated before the pipette touched the cell. After a gigaseal (>10 GΩ) was obtained, the cell membrane was ruptured by gentle suction to establish the whole cell configuration.

The series resistance (Rs) was electrically compensated to minimize the capacitive surge on the current recording and the voltage drop across the clamped membrane. Rs along the clamp circuit was estimated by dividing the capacitive time constant (obtained by fitting the decay of the capacitive transient) by the calculated membrane capacitance (the time integral of the capacitive response to a 5-mV hyperpolarizing pulse from a holding potential of −60 mV divided by the voltage drop). Membrane capacitance was 1.79 ± 0.10 pF (n = 20). To correct for differences in cell size, all membrane data are expressed as current densities (i.e., normalized to capacitance). Before compensation, the capacitive time constant and Rs, averaged 1.314 ± 113.9 μs and 8.6 ± 0.5 MΩ, and after compensation corresponding values were 313.3 ± 26.2 μs and 2.0 ± 0.3 MΩ. ICa rarely exceeded 2 nA, and the mean maximum voltage drop across the Rs therefore, did not exceed 4 mV. Cells with significant leak current were rejected.

The liquid junction potentials between the external and pipette solutions were 10–11 mV and were not corrected.

The average length of single human ventricular myocytes was 148.3 ± 8.8 μm (n = 15, range 112–185 μm), and the diameter was 18.5 ± 1.2 μm (range 12–25 μm); the estimated cell surface area was therefore 9.2 ± 0.5 × 10^-6 cm^2 on the basis of assumed right cylinder geometry. The input resistance (Ri), in series with Rs, was measured by using four consecutive 5-mV hyperpolarizing steps from a holding potential of −60 mV, with the resulting change in current used to calculate Ri, (16).

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Mean Rs, as estimated in 11 cells was 56.7 ± 5.4 MΩ. The resting space constant (sc) was estimated as follows: sc = \sqrt{r(Rs/2)}

where r is the cell radius, Rs is the specific membrane resistance, and Ri is intracellular resistivity. Rs was estimated from the product of Rs, and surface area, providing a mean value of 5.1 ± 0.2 KΩ·cm^2, and Ri was assumed to be 100–200 Ω·cm (16, 34, 40). The mean values of sc were 1.54 ± 0.22 and 1.09 ± 0.16 mm, which are more than seven times the single cell length. These could be underestimated, since the surface area estimated on the basis of a specific capacitance of 1 µF/cm^2 is 17.9 × 10^-5 cm^2, twice as large as the value above, which indicates that membrane infolding results in a true surface area larger than that of a right cylinder (40).

Cells were current clamped to record action potentials and/or voltage clamped with rectangular steps or individual action potential waveforms at 0.5 Hz with pClamp6. In the action potential clamp experiments, ICa was identified by subtracting currents before and after the substitution of external Ca^{2+} with equimolar Mg^{2+}, as described by Bouchard et al. (9). Similar results were obtained with use of Cd^{2+} (200 µM) to suppress ICa, but all the results were with Mg^{2+} substitution, because Cd^{2+} can also affect Na+ current (INa).

Nonlinear curve-fitting techniques (Clampfit in pClamp6 or Sigmaplot, Jandel Scientific, San Rafael, CA) based on the Marquardt procedure were used to fit equations to experimental data. Paired and nonpaired Student’s t-tests were used as appropriate to evaluate the statistical significance of differences between two group means, and ANOVA was used for multiple groups. P < 0.05 was considered to indicate significance. Group data are expressed as means ± SE.

RESULTS

Relation between changes in APD and ICa. To study the relation between rate-dependent abbreviation of APD and ICa, we recorded action potentials in current-clamp mode at 0.5, 1, and 2 Hz before and after external Ca^{2+} substitution with equimolar external Mg^{2+}. The resting membrane potentials were between −69 and −73 mV without any artificial current injection. Figure 1A shows action potentials recorded from a representative human ventricular myocyte at frequencies of 0.5, 1, and 2 Hz in the presence of 2 mM external Ca^{2+} (control). APD decreased as stimulus rates increased. The average changes in APD at 50 and 90% repolarization (APD50 and APD90) are shown in Fig. 1B. APD50 and APD90 decreased from 325 ± 23 and 439 ± 37 ms, respectively, at a frequency of 0.5 Hz to 181 ± 12 and 281 ± 21 ms, respectively, at 2 Hz (P < 0.01, n = 18).

The mean rate-dependent reductions were 44 ± 4% for APD50 and 36 ± 4% for APD90. Replacement of external Ca^{2+} by 2 mM external Mg^{2+} greatly attenuated APD adaptation to rate. Figure 1C shows APD recordings from the same cell and at the same frequencies as in Fig. 1A but in the presence of 2 mM external Mg^{2+} to replace external Ca^{2+}. APD was substantially reduced and showed little alteration with changes in rate. As indicated in Fig. 1C, inset, substitution of external Ca^{2+} with external Mg^{2+} totally suppressed ICa on depolarization from −40 to 0 mV in the same cell. In eight cells, replacement of external Ca^{2+} virtually abolished rate-dependent changes in APD, as shown by the mean data in Fig. 1D. The reduction in APD50 and APD90 caused by increasing rate from 0.5 to 2 Hz averaged 5 ± 2 and 6 ± 3%, respectively, which is substantially less (P < 0.01) than under control conditions over the same range of frequencies. On restoration of external Ca^{2+} concentration to 2 mM, rate-dependent changes in APD50 and APD90 were restored to 39 ± 5 and 33 ± 5% as rate increased from 0.5 to 2 Hz (n = 4).

Action potential clamp and ICa. To obtain more information about how ICa contributes to rate-dependent...
abbreviation of APD, we used the action potential waveform to clamp individual cells and to quantify $I_{Ca}$ during the action potential at different frequencies. The action potential was acquired from each cell at 0.5 Hz and used as a voltage-clamp waveform in the same cell. External Ca$^{2+}$-dependent current ($I_{Ca}$) was measured by digital subtraction of the currents before and after substitution of external Ca$^{2+}$ with equimolar external Mg$^{2+}$, as described by Bouchard et al. (9). Figure 2 shows representative recordings from a ventricular myocyte. Figure 2A demonstrates the action potential waveform, and Fig. 2B shows currents elicited by the action potential waveform in the presence (control) and absence of 2 mM external Ca$^{2+}$ (Mg$^{2+}$). Figure 2C shows the $I_{Ca}$ obtained by subtracting currents of Fig. 2B in the absence of external Ca$^{2+}$ from those in its presence (control). $I_{Ca}$ increased rapidly to a peak and then decreased rapidly to a plateau, which terminated with repolarization.

Figure 3 displays external Ca$^{2+}$-dependent charge movement during the action potential at 0.5–2 Hz in a representative human ventricular myocyte. Figure 3A shows the action potential waveform, and Fig. 3B displays current recordings obtained by digital subtraction from control currents (in the presence of 2 mM Ca$^{2+}$) at 0.5, 1, and 2 Hz of the currents recorded at corresponding frequencies in the presence of 2 mM external Mg$^{2+}$ (to replace external Ca$^{2+}$). External Ca$^{2+}$-dependent $I_{Ca}$ magnitude was significantly decreased at 1 and 2 Hz, compared with 0.5 Hz, during the action potential.
Peak and plateau $I_{Ca}$ were evaluated during the action potential at the frequencies tested. Plateau $I_{Ca}$ was measured at 150 ms from action potential depolarization. In a total of nine cells, peak and plateau $I_{Ca}$ substantially decreased as frequency increased. Peak and plateau $I_{Ca}$ densities declined from 5.6 ± 0.5 and 1.7 ± 0.3 pA/pF at 0.5 Hz to 3.7 ± 0.3 and 1.1 ± 0.2 pA/pF at 2 Hz (P < 0.01). The results from action potential clamp support the concept that the rate-dependent abbreviation of APD is related to the reduction of Ca$^{2+}$ influx during the action potential at higher frequencies.

To further study $I_{Ca}$ during the action potential, we applied the action potential waveforms recorded at 0.5 and 2 Hz. Table 1 shows peak and plateau $I_{Ca}$ at 150 ms from action potential depolarization with the two action potential templates in a total of five cells. No significant difference was observed in peak $I_{Ca}$ density between 0.5- and 2-Hz waveforms, and plateau $I_{Ca}$ was significantly smaller in the 2- than in the 0.5-Hz action potential waveform at 0.5 and 2 Hz. Although a similar ratio of 2 to 0.5 Hz for peak and plateau $I_{Ca}$ was observed with the two templates, the results indicate the importance of $I_{Ca}$ for maintaining action potential.

Frequency-dependent reduction of $I_{Ca}$. To study why Ca$^{2+}$ influx decreased during the action potential when the rate increased, conventional step voltage-clamp protocols were used to determine use and frequency dependence of $I_{Ca}$ under conditions designed to suppress other currents (K$^+$ replacement by Cs$^+$ in the pipette and extracellular Na$^+$ replacement by choline).

Figure 4 shows the use and frequency dependence of $I_{Ca}$ under these conditions. $I_{Ca}$ was recorded with trains of 15 pulses (300 ms from −80 to +10 mV) at 0.2, 0.5, 1, and 2 Hz (60 s between trains). Figure 4A displays representative current traces from the 1st and 15th pulses at 2 Hz. $I_{Ca}$ was clearly smaller during the 15th than during the 1st pulse, and time-dependent inactivation of $I_{Ca}$ appeared faster at the 15th than that at the 1st pulse. Figure 4B shows changes in $I_{Ca}$ during each beat expressed as a function of the first pulse at each frequency. Statistically significant use dependence was noted at frequencies >0.2 Hz (P < 0.05, 0.01, and 0.01 for 0.5, 1, and 2 Hz, respectively). The use-dependent reductions in $I_{Ca}$ resulted in frequency dependence of the current, as shown in Fig. 4C, which illustrates the change in relation between pulse frequency and steady-state peak $I_{Ca}$ with currents of each frequency normalized to values at 0.2 Hz. $I_{Ca}$ was reduced significantly at each frequency, with a reduction of 36 ± 2% at 2 Hz.

Time-dependent reactivation of $I_{Ca}$. The time dependence of $I_{Ca}$ reactivation was studied with the paired-pulse protocol illustrated in Fig. 5. Identical 300-ms pulses (P1 and P2) from a holding potential of −80 to +10 mV were delivered every 10 s, with varying P1-P2 intervals. The current during P2 relative to the current during P1 was determined as a function of the P1-P2 reactivation interval (Fig. 5A). The curves in Fig. 5B show nonlinear curve fits to mean data at holding potentials of −80 mV (n = 11) and −60 mV (n = 7). $I_{Ca}$ recovery reached 97% and was well fit by a biexponential function with time constants of 65 ± 12 and 638 ± 39 ms for a holding potential of −80 mV and 164 ± 15 and 697 ± 45 ms for a holding potential of −60 mV.

Current-voltage relation of $I_{Ca}$. Two types of $I_{Ca}$ ["T type" ($I_{CaT}$) and "L type" ($I_{CaL}$)] have been found in cardiac cells from a variety of species (4, 5, 30, 38). To determine whether $I_{CaT}$ is present in human ventricular cells and contributes to rate-dependent changes in APD, current-voltage (I-V) relations of $I_{Ca}$ were deter-

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**Table 1.** Peak and plateau $I_{Ca}$ (at 150 ms from action potential depolarization) during action potential

<table>
<thead>
<tr>
<th>AP Waveform</th>
<th>0.5 Hz</th>
<th>2 Hz</th>
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<tr>
<td></td>
<td>Peak $I_{Ca}$</td>
<td>Plateau $I_{Ca}$</td>
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<tr>
<td>0.5 Hz</td>
<td>5.2 ± 0.5</td>
<td>1.5 ± 0.3</td>
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<tr>
<td>2.0Hz</td>
<td>3.5 ± 0.4*</td>
<td>1.0 ± 0.2*</td>
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<tr>
<td>Ratio (2/0.5)</td>
<td>0.673</td>
<td>0.666</td>
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Values are means ± SE in pA/pF; n = 5. $I_{Ca}$, Ca$^{2+}$ current; AP, action potential. *P < 0.01 vs. rate at 0.5 Hz; †P < 0.05 vs. 0.5-Hz AP waveform.
mined using 300-ms depolarizing steps every 10 s from holding potentials of −80 and −40 mV. The magnitude of \( I_{\text{Ca}} \) was measured as the difference between the peak inward current and the steady-state current at the end of the depolarizing step. For analyses of average currents, \( I_{\text{Ca}} \) was normalized to cell capacitance to control for variations in cell size.

Figure 6A shows \( I_{\text{Ca}} \) recordings from a representative myocyte at a holding potential of −80 mV in the absence and presence of the L-type Ca\(^{2+}\) channel blocker nifedipine (10 μM). Nifedipine abolished the membrane current, which suggests that the current elicited by the voltage protocol shown is \( I_{\text{Ca,L}} \). Figure 6B shows mean \( I_{\text{Ca}} \) density-voltage relations. \( I_{\text{Ca}} \) density was significantly less at −40 mV (\( n = 10 \)) than at −80 mV (\( n = 12 \)) over a broad range of test potentials. Maximum current density was obtained at the same voltage (+10 mV) at each holding potential. No evidence for \( I_{\text{Ca,T}} \), in terms of a discrete component that activates and inactivates at more negative voltages (4, 5, 30, 31), was observed in any cell.

Voltage-dependent activation and inactivation of \( I_{\text{Ca}} \). The voltage dependence of \( I_{\text{Ca}} \) activation can be determined from the I-V relation of \( I_{\text{Ca}} \) on the basis of the following formulation: \( \Delta I_{\text{d}} = \Delta I_{\text{a}}/G_{\text{a}}(V_{\text{t}} - V_{\text{r}}) \), where \( \Delta I_{\text{d}} \) is the effective variable and \( I_{\text{a}} \) is \( I_{\text{Ca}} \) at a test potential \( V_{\text{t}} \), \( G_{\text{a}} \) is the maximum conductance, and \( V_{\text{r}} \) is the reversal potential, estimated from a linear fit of the terminal portion of the ascending limb of the I-V relation of \( I_{\text{Ca}} \). We used this equation to calculate the \( I_{\text{Ca}} \) activation variable from the I-V relation at a holding potential of −80 mV (Fig. 6B). Values of \( \Delta I_{\text{d}} \) were normalized to the maximum value in each cell to obtain the activation variable.

The voltage protocol and representative recordings used to assess \( I_{\text{Ca}} \) inactivation are illustrated in Fig. 7A. Prepulses of 400-ms duration were applied to conditioning potentials between −100 and +60 mV, and then \( I_{\text{Ca}} \) was recorded during a 300-ms test pulse to +10 mV. The inactivation variable (f) was determined as \( I_{\text{Ca}} \) at a given prepulse potential divided by the maximum \( I_{\text{Ca}} \) in the absence of a prepulse.

Figure 7B shows the results obtained from the analysis of voltage-dependent activation and inactivation described above. Mean data are shown by the symbols, and the curves shown are best-fit Boltzmann distributions. The half-activation voltage (\( V_{0.5} \)) averaged −4.8 ± 0.9 mV (\( n = 10 \)), and the slope factor was 6.2 ± 1.1 mV. Inactivation reached a maximum at +10 mV and was incomplete. At more positive voltages, the extent of inactivation decreased. The inactivation curve was nonmonotonic or “U shaped,” compatible with an important Ca\(^{2+}\)-dependent component to inactivation previously seen in human atrial cells (12, 25), and could be fit by a Boltzmann relation with the following equation (33, 34): \( f = 1/(1 + \exp[(V - V_{0.5})/K]) + R \), where \( V_{0.5} \) is the estimated half-maximum inactivation voltage, \( V \) is prepulse potential, \( K \) is the slope factor, and \( R \) charac-
Fig. 7. Voltage-dependent activation and inactivation of I_{Ca}.
A: representative current recordings used to determine voltage dependence of I_{Ca} inactivation. Cell was depolarized by 400-ms prepulses from HP of −80 mV to between −100 and 0 mV and back to −80 mV for 5 ms, then subjected to a 300-ms test pulse to +10 mV. B: mean voltage-dependent activation and inactivation relations for I_{Ca}. Inactivation was assessed with protocol shown in A. Data were fit to Boltzmann relations for activation and inactivation as follows: d = 1/[1 + exp((V_{0.5} − V)/K)] and f = 1/[1 + exp((V − V_{0.5})/K)] + R, respectively, where V is membrane potential, V_{0.5} is membrane potential for half-maximal activation or inactivation, K is a slope factor, and R is a term to characterize degree of incomplete inactivation (see RESULTS) at positive voltages. Values of d and f were calculated as described in RESULTS.

Fig. 8. Rate-dependent slow component of delayed rectifier K^+ current (I_{Ks}). A: voltage protocol used to determine rate-dependent I_{Ks} and tail current. B: I_{Ks} and tail current activated by a ramp protocol superimposed at 0.5 and 3.33 Hz in a representative human ventricular cell. Experiment was conducted with a K^+ and Na^+-free external solution in presence 5 μM E-4031, 5 mM 4-aminopyridine, and 200 μM Cd^2+. Similar results were obtained in a total of 4 cells.

DISCUSSION

We have demonstrated that rate-dependent abbreviation of APD in human ventricular myocytes is closely related to changes in Ca^{2+} influx during the action potential caused by frequency-dependent reduction of I_{Ca}, due, at least in part, to incomplete time-dependent recovery at higher frequencies.

Comparison with previously published studies of mechanisms for rate-dependent changes of APD in cardiac cells. Rate-dependent abbreviation in cardiac APD has been considered to be related to an increase in transmembrane K^+ conductance (22) and/or an increase in Na^+-K^+ pump activity (11) on the basis of the observation that extracellular K^+ was elevated with a parallel increase in frequency (21, 22). Transient outward K^+ current (I_{to}) has been described in human ventricular cells (8, 23, 41); however, its property of rate-dependent reduction does not account for the increase in extracellular K^+ or for rate-dependent reduction in APD. Inward rectifier background current (I_{K1}) has been reported to show a rate-dependent decrease in guinea pig ventricular cells that is dependent on the presence of I_{Ca} (13), but a decrease of the outward current would not account for rate-dependent abbreviation of APD.

I_{Ks} has been reported to show a rate-dependent increase in guinea pig ventricular cells and to contribute to APD abbreviation in that species (20). I_{Ks} and tail current have been described in human ventricular cells (24); however, I_{Ks} is much smaller in human than in guinea pig ventricular cells. With the use of a ramp protocol, a decrease in I_{Ks} or tail current was observed, perhaps because I_{Ks} is so small that the rate-dependent change in human ventricular cells could not be seen.

TERMINAL

The degree of incomplete inactivation (R = 0.18/[1 + 10 exp((V_{0.5} − V)/K)]) provides the curve shown in Fig. 7B. V_{0.5} averaged −28.5 ± 2.8 mV (n = 7), and K averaged 7.8 ± 1.4 mV.

Rate-dependent I_{Ks}. Because a rate-dependent increase in I_{Ks} is believed to contribute to the shortening of APD at high rates in guinea pig ventricular myocytes (20), we determined a possible effect of changing depolarization rates on I_{Ks} in human ventricular cells. I_{Ks} was examined by a 2-s ramp protocol from −50 to +40 mV after a train (0.5 or 3.33 Hz) of 30 step voltage pulses (500-ms; Fig. 8A). The experiment was performed with an external solution that was free of Na^+ and K^+ in the presence of 5 μM E-4031 [to block the rapid component of the delayed rectifier K^+ current (I_{Kr})], 5 mM 4-aminopyridine [to block transient outward K^+ current (I_{to}), and 200 μM Cd^2+ (to block I_{Ca}). Figure 8B shows the ramp-activated I_{Ks} with small tail current superimposed at 0.5 and 3.33 Hz in a representative human ventricular cell. Similar results were observed in a total of four cells. No significant differ-
clear rate-dependent increase in \( I_{\text{Ks}} \) was not observed in human ventricular cells with an increase in frequency from 0.5 to 3.3 Hz (Fig. 8), perhaps because the \( I_{\text{Ks}} \) is so small that the increase at a high rate could not be seen. The small \( I_{\text{Ks}} \) in human ventricular cells we reported previously (24) and recorded in this study may be due to an artifact of the cell isolation procedure and/or the reduced \( I_{\text{Ks}} \) expression in myocytes from failing hearts (but not in normal myocytes).

The present study reveals a close relation between the rate-dependent abbreviation of APD and reduction of Ca\(^{2+} \) influx during the action potential. Earlier studies demonstrate that \( I_{\text{Ca}} \) is very important for determining the plateau phase of cardiac action potential (6), and \( I_{\text{Ca}} \) blockade dramatically shortens cardiac APD (19), whereas \( I_{\text{Ca}} \) increases induced by β-adrenergic stimulation (25, 39) and Ca\(^{2+} \) channel agonists (39) prolong APD in myocardium, supporting the findings of the present study.

Comparison with previously published studies of \( I_{\text{Ca}} \) in human cardiac cells. Several groups have described the properties of \( I_{\text{Ca}} \) in human cardiac cells (14, 26, 28, 29). Our study differs from these in that we determined Ca\(^{2+} \) influx during the action potential and recorded \( I_{\text{Ca}} \) at body temperature (all previous studies in human ventricular myocytes have been performed at room temperature), characterized recovery and frequency-dependent properties in detail (not reported in previous work), and evaluated the role of \( I_{\text{Ca}} \) in human ventricular action potential behavior (not previously performed). Previous studies have not examined the presence and amplitude of \( I_{\text{Ca,T}} \) in the human ventricle. In the present study we did not find evidence for \( I_{\text{Ca,T}} \) in human ventricular myocytes.

We found that \( I_{\text{Ca}} \) recovery from steady-state inactivation is biexponential, with time constants of 65 and 683 ms at -80 mV and 164 and 697 ms at -60 mV, which indicates that slight membrane depolarization may slow \( I_{\text{Ca}} \) recovery. We also found that \( I_{\text{Ca}} \) shows significant steady-state inactivation at 1 Hz. These findings imply that \( I_{\text{Ca}} \) is partially inactivated at normal heart rates (70 beats/min) and that physiological increases in heart rate are likely to cause further \( I_{\text{Ca}} \) inactivation.

The time dependence of \( I_{\text{Ca}} \) reactivation in ventricular cells is different from our previous findings in human atrial myocytes, in which \( I_{\text{Ca}} \) reactivation is monoexponential at -80 mV, with a time constant of 56 ms. Correspondingly, the frequency-dependent decrease in \( I_{\text{Ca}} \) at -80 mV was more important in ventricular cells (35% reduction at 2 Hz) than in human atrial cells (10% reduction at 2 Hz) (25). Therefore, changes in \( I_{\text{Ca}} \) would contribute more to rate-dependent abbreviation of APD in ventricular cells than in atrial cells in humans. We were unable to find reports of \( I_{\text{Ca}} \) frequency dependence in human ventricular myocytes with which to compare our findings.

Significance of our observations. A better understanding of the ionic mechanisms governing human ventricular repolarization is important for designing improved antiarrhythmic strategies. The rate-dependent properties of ventricular APD and refactoriness are known to be an important determinant of the occurrence of reentrant cardiac arrhythmias (36). Attuel et al. (2) showed that refractoriness abbreviation with increased rates characterizes patients with vulnerability to atrial reentrant arrhythmias. The present study suggests that frequency-dependent \( I_{\text{Ca}} \) reduction contributes significantly to frequency-dependent ventricular APD abbreviation in humans. There is considerable interest in developing novel antiarrhythmic drugs that act selectively during tachycardia, and a better understanding of the mechanisms of physiological APD adaptation to tachycardia in humans, as developed in the present study, is important for this effort.

The properties of \( I_{\text{Ca}} \) have been well described in animals with the use of conventional rectangular depolarizing clamp steps. Little direct information is available in the literature regarding the \( I_{\text{Ca}} \) contribution to rate-dependent changes in APD. Bouchard et al. (9) employed the action potential clamp technique to determine the effects of APD change on excitation-contraction coupling in rat ventricular myocytes. They evaluated \( I_{\text{Ca}} \) during an action potential by subtracting membrane currents before and after the addition of 100 µM Cd\(^{2+} \) or after the substitution of external Ca\(^{2+} \) with external Mg\(^{2+} \). They quantified \( \int I_{\text{Ca}} \) in rat ventricular myocytes during action potentials and showed that \( I_{\text{Ca}} \) was inactivated before the action potential terminated in rat ventricular cells (9). In the present study we applied the action potential clamp to examine how much \( I_{\text{Ca}} \) was present during the plateau of the action potential, by subtracting membrane currents before and after the substitution of external Ca\(^{2+} \) with external Mg\(^{2+} \) in human ventricular myocytes, and found considerable inward \( I_{\text{Ca}} \) during the action potential plateau. Tachycardia reduced plateau \( I_{\text{Ca}} \), accounting for action potential abbreviation. Reduced APD during tachycardia shortens the refractory period and the wavelength and may promote the occurrence of reentrant arrhythmias.

Potential limitations. We studied right ventricular cells from patients with severe left ventricular failure, and the patients were receiving medications including captopril, digoxin, dobutamine, and furosemide. It is likely that medications were washed out in the cell isolation process, but we cannot fully exclude an effect of medication on our results. Although the cells used in this study were from hearts that were assessed by the pathologist to have relatively mild or no changes in the right ventricular myocardium, we cannot exclude the possibility that our results were affected by the presence of heart disease. The small \( I_{\text{Ks}} \) we recorded may be related to the reduced expression during heart disease.

To study the effect of \( I_{\text{Ca}} \) on rate-dependent changes in APD, \( I_{\text{Ca}} \) must be blocked; however, pure Ca\(^{2+} \) channel blockers are not available. The inorganic Ca\(^{2+} \) channel blocker Cd\(^{2+} \) can affect \( I_{\text{Na}} \), whereas the 1,4-dihydropyridine Ca\(^{2+} \) antagonists may also block \( I_{\text{to}} \) (17). We therefore replaced external Ca\(^{2+} \) with equimolar Mg\(^{2+} \) (9). The elimination of \( I_{\text{Ca}} \) may limit Ca\(^{2+} \)-induced Ca\(^{2+} \) release from sarcoplasmic reticulum and, therefore, inhibit Na\(^+\)/Ca\(^{2+} \) exchanger current.
during the action potential (7). This may also induce action potential shortening, which may cause an overestimation of the direct role of I_{Ca} in action potential abbreviation. On the other hand, possible Ca^{2+}-activated currents, such as Ca^{2+}-activated Cl\^- and K\^+ currents, are also reduced by the removal of external Ca^{2+}, but the effect would not account for rate-dependent shortening of APD, since Ca^{2+}-activated Cl\^- and K\^+ currents are repolarizing currents, which will tend to shorten APD.

In the present study, I_{Ca} and I_{Ca} kinetics were studied using Na\^-free superfusate to prevent contamination by I_{Na}. In the absence of extracellular Na\^+, Ca^{2+} extrusion via the Na\^+/Ca^{2+} exchanger is inhibited and cellular integrity is threatened by progressive Ca^{2+} overload. Cells were therefore dialyzed with 10 mM EGTA via the pipette solution. However, this may result in strong intracellular Ca^{2+} buffering, limit I_{Ca} inactivation because of sarcoplasmic reticulum Ca^{2+} release, and slow the rapid phase of inactivation (37). Therefore, we may have underestimated the rate of this process and the extent of physiological frequency-dependent I_{Ca} inactivation.

I_{Ca} rundown may be problematic when patch-clamp techniques are used. In our experience, cell quality is a major factor determining rundown, and we did not observe important rundown over the course of our experiments. We recorded current amplitude before and after each protocol. If maximum current amplitude decreased by >5% over the course of an experimental protocol, the data were discarded.

At room temperature, upregulation of I_{Ca} has been reported at high frequency in atrial myocytes from a subgroup of patients (32). We did not find such an increase, perhaps because of differences in experimental protocols and conditions, cell types, and/or patient population.

In conclusion, we have demonstrated that rate-dependent abbreviation of human ventricular APD is largely attributable to rate-dependent changes in I_{Ca}. Time-dependent recovery of I_{Ca} and its consequent frequency dependence are likely to be significant contributors to the physiological control of human ventricular APD and to be important in controlling the occurrence of ventricular reentrant arrhythmias such as ventricular tachycardia and/or fibrillation.

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