Ionic Mechanism of Delayed Afterdepolarizations in Ventricular Cells Isolated From Human End-Stage Failing Hearts

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Background—Animal studies have shown that the Ca2+-activated Cl− current (I_{Cl(Ca)}) and the Na+/Ca2+ exchange current (I_{Na/Ca}) contribute to the transient inward current (I_{ti}). I_{ti} is responsible for the proarrhythmic delayed afterdepolarizations (DADs). We investigated the ionic mechanism of I_{ti} and DADs in human cardiac cells.

Methods and Results—Human ventricular cells were enzymatically isolated from explanted hearts of patients with end-stage heart failure and studied with patch-clamp methodology. I_{ti} were elicited in the presence of 1 μmol/L norepinephrine by trains of repetitive depolarizations from −80 to +50 mV. DADs were induced in the presence of 1 μmol/L norepinephrine at a stimulus frequency of 1 Hz. I_{ti} currents were inwardly directed over the voltage range between −110 and +50 mV. Neither the Cl− channel blocker 4,4′-disothiocyanostilbene-2,2′-disulfonic acid nor changes in [Cl−], affected I_{ti} or DAD amplitude. This excludes an important role for I_{Cl(Ca)}. Blockade of Na+/Ca2+ exchange by substitution of all extracellular Na+ by Li+-, conversely, completely inhibited I_{ti}. In rabbit, I_{Cl(Ca)} density in ventricular cells isolated from control hearts did not differ significantly from that in ventricular cells isolated from failing hearts.

Conclusions—In contrast to many animal species, I_{ti} and DADs in human ventricular cells from failing hearts consist only of I_{Na/Ca}. In rabbits, heart failure per se does not alter I_{Cl(Ca)} density, suggesting that I_{Cl(Ca)} may also be absent during DADs in nonfailing human ventricular cells. (Circulation. 2001;104:2728-2733.)

Key Words: arrhythmia ■ heart failure ■ ion channels ■ sodium ■ calcium
question the rationale behind $I_{\text{Cl(Ca)}}$ blockade as a therapeutic approach in arrhythmias caused by triggered activity based on DADs.

**Methods**

**Cell Preparation**

**Human Ventricular Cells**

Hearts were obtained from patients with end-stage heart failure caused by either ischemic or dilated cardiomyopathy. Patient characteristics are shown in the Table. All patients were in NYHA functional class IV and received standard therapy for chronic heart failure. Informed consent was obtained before heart transplantation, and the protocol complied with institutional guidelines. Ventricular cells were isolated from the left ventricle by enzymatic dissociation.

**Rabbit Ventricular Cells**

Heart failure was induced in New Zealand White rabbits by volume and pressure overload. The heart failure index based on relative heart weight, relative lung weight, left ventricular end-diastolic pressure, third heart sound, and ascites was calculated as described previously. We used only hearts of rabbits in which 4 of the aforementioned 5 parameters were abnormal. Age-matched animals served as control group. Animal care was in accordance with institutional guidelines. Cells from the left ventricle were isolated by enzymatic dissociation. Cells isolated from failing hearts were served as control group. Animal care was in accordance with institutional guidelines.

**Recording Procedures**

Small aliquots of cell suspension were placed in a recording chamber on the stage of an inverted microscope and superfused with Tyrode’s solution (35°C to 37°C) containing (mmol/L) NaCl 140, KCl 5.4, CaCl₂ 1.8, MgCl₂ 1.0, glucose 5.5, and HEPES 10 (pH adjusted to 7.2 with NaOH).

Membrane potentials and currents were recorded in the ruptured-patch whole-cell configuration of the patch-clamp technique. Patch pipettes (3 to 5 MΩ) were pulled from borosilicate glass and filled with solution containing either (mmol/L) potassium gluconate 125, KCl 20, and HEPES 10 (pH adjusted to 7.2 with KOH) or KCl 145 and HEPES 10 (pH adjusted to 7.2 with KOH). Potentials were corrected for the liquid junction potential. Membrane currents and potentials were low-pass filtered online with a cutoff frequency of 1 kHz and digitized at 2 kHz.

**Stimulation Protocols**

DADs and $I_{\text{KS}}$ result from spontaneous SR Ca$^{2+}$ release in Ca$^{2+}$-overloaded myocytes. In our experiments, we induced sarcoplasmic Ca$^{2+}$ overload by application of 1 μmol/L norepinephrine (Centrafarm). DADs were induced by eliciting action potentials (1 Hz) with current pulses applied through the patch pipette. $I_t$ was elicited by repeated trains of 15 to 20 200-ms voltage-clamp steps from −80 to +50 mV (Figure 1A). Time between the steps was 100 ms. Successive trains were 6 seconds apart.

During the first 5 to 6 successive voltage-clamp steps of a train, both the quasi steady-state current during the 200-ms depolarizing step and the inwardly directed “tail” current during the 100-ms repolarizing step increase until they reach a stable value (Figure 1B). Enhanced delayed rectifier current ($I_{\text{Kd}}$) and $I_{\text{NaCa}}$ may underlie this phenomenon. Both $I_t$ and $I_{\text{NaCa}}$ increase during a rise in [Ca$^{2+}$]i. As occurs during the train of voltage-clamp steps, $I_{\text{NaCa}}$ results from repeated activation of Ca$^{2+}$ current and the diminished Ca$^{2+}$ extrusion by the Na$^+$/Ca$^{2+}$ exchanger. Slow inactivation kinetics of $I_t$ presumably also plays a role in the increase of the quasi steady-state current.

After cessation of a train, all cells develop $\pm I_t$ oscillations, typically within 3 seconds (Figure 1B, arrow). Every $I_t$ was accom-

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**Patient Data**

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<th>Patient</th>
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<td>Antico, ACE, nit, diu, dig, Ca</td>
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EF indicates ejection fraction; ICM, ischemic cardiomyopathy; DCM, dilated cardiomyopathy; VF, ventricular fibrillation; ICD, internal cardioverter defibrillator; VT, sustained ventricular tachycardia; nsVT, nonsustained ventricular tachycardia; antico, anticoagulants; ACE, ACE inhibitors; nit, nitrates; diu, diuretics; dig, digoxin; Ca, Ca antagonists; dop, dopamine; and ami, amidarone.
Results

In Human Ventricular Cells, \( I_{\text{ti}} \) and DADs Are Caused by Spontaneous SR \( \text{Ca}^{2+} \) Release

We first asked the question whether in human ventricular cells \( I_{\text{ti}} \) and DADs are activated by spontaneous SR \( \text{Ca}^{2+} \) release. We tested the effects of 0.2 mmol/L tetracaine and 5 mmol/L caffeine, drugs known to inhibit spontaneous SR \( \text{Ca}^{2+} \) release.6,21 Both tetracaine (Figure 1C, n=3) and caffeine (n=4, data not shown) completely abolished \( I_{\text{ti}} \) and DAD. From these experiments, we conclude that as in animal cells, in human ventricular cells \( I_{\text{ti}} \) and DADs also are caused by spontaneous SR \( \text{Ca}^{2+} \) release.

No Role for a Cl\(^{-}\) Current in \( I_{\text{ti}} \) and DAD in Human Ventricular Cells

Next, we investigated the currents contributing to \( I_{\text{ti}} \) and DAD in human ventricular cells. We determined the current-voltage (I-V) relationship of \( I_{\text{ti}} \) in human ventricular cells recorded with pipette solution containing high (n=4; \( E_{\text{Cl}}=-50 \text{ mV} \)) and low (n=5; \( E_{\text{Cl}}=-50 \text{ mV} \)) \( \text{Cl}^{-} \)-sensitive \( \text{Ca}^{2+} \) channels. 23 In these experiments, we tested and had a maximal amplitude at approximately 70 mV. The 2 \( I_{\text{ti}} \) I-V relationships did not differ significantly. In accordance with this observation, we also found no significant effect of \([\text{Cl}^{-}]_{\text{i}}\) on DAD amplitude (Figure 2C). In fact, resting membrane potential and the duration and amplitude of the action potential also were not affected. From these experiments, we conclude that it is not likely that \( I_{\text{Cl(Ca)}} \) contributes much to \( I_{\text{ti}} \)s and DADs in failing human ventricular cells.

To substantiate this conclusion, we studied the effects of \( I_{\text{Cl(Ca)}} \) blockade on the \( I_{\text{ti}} \) I-V relationship and DAD amplitude. We applied DIDS, a potent inhibitor of anion transport proteins, including \( I_{\text{Cl(Ca)}} \) channels.23 In these experiments, we used the low-\( \text{Cl}^{-} \) pipette solution (\( E_{\text{Cl}}=-50 \text{ mV} \)). Figure 3A shows typical current traces recorded at −80 and +50 mV in absence and presence of 0.5 mmol/L DIDS. B, I-V relationship of \( I_{\text{ti}} \) in absence and presence of 0.5 mmol/L DIDS (n=4). C, Typical action potential and DAD configuration in absence and presence of 0.5 mmol/L DIDS. D, Action potential and DAD parameters in absence and presence of 0.5 mmol/L DIDS (n=4). Abbreviations as in Figure 2.
Figure 3B shows the lack of effect of the stilbene on the mean $I_h$ $I$-V relationship of 4 cells. Figure 3C and 3D shows that DIDS also had no effect on DAD amplitude or other action potential characteristics ($n$=4).

From these experiments, we conclude that there is no or only a very limited role for a Cl$^-$ current in DADs and $I_h$ in failing human ventricular cells.

Dominant Role for $I_{NaCa}$ in $I_h$ and DAD Formation in Human Ventricular Cells

A salient feature of the $I_h$ $I$-V relationship is the absence of a reversal potential (Figures 2B and 3B). This suggests that not a channel mechanism but rather an electrogenic Na$^+$/Ca$^{2+}$ exchange underlies $I_h$. Na$^+$/Ca$^{2+}$ exchange can support inwardly and outwardly directed currents.24 During spontaneous SR Ca$^{2+}$ release under voltage-clamp conditions, however, $I_{NaCa}$ does not reverse sign and remains inwardly directed, because any rise in sarcoplasmic Ca$^{2+}$ concentration will shift the reversal potential of the exchanger toward more positive membrane potentials.25,26 The $I_h$ in human ventricular cells was small at positive membrane potentials and increased at more hyperpolarized potentials, with a maximum at $-70$ mV. At potentials negative to $-70$ mV, $I_h$ decreases (Figures 2B and 3B). This behavior is most likely related to voltage-dependency of $I_{NaCa}$ itself, but also to the amount of Ca$^{2+}$ released by the SR.24,27 Spontaneous [Ca$^{2+}$]$_i$ oscillations seem to decrease at more hyperpolarized potentials,28 which will result in a smaller $I_h$.

Next, we tested the hypothesis that $I_{NaCa}$ contributes to $I_h$. We inhibited Na$^+$/Ca$^{2+}$ exchange by replacing extracellular Na$^+$ with equimolar amounts of Li$^+$. Li$^+$ permeates through Na$^+$ channels and nonselective cation channels28 but cannot replace Na$^+$ on the Na$^+$/Ca$^{2+}$ exchanger.29,30 Throughout these experiments, 0.5-mmol/L DIDS was present, and the low-Cl$^-$ pipette solution (E$_{Cl}$ = $-50$ mV) was used. Figure 4A shows a typical example of the effects of Li$^+$ on $I_h$. Both at $-80$ and at $+50$ mV, an inwardly directed $I_h$ was found (left), which completely disappeared after substitution of Li$^+$ for Na$^+$ (right). Aftercontractions were still visible, however, indicating that spontaneous SR Ca$^{2+}$ release was not dis-

In a Rabbit Model, $I_{Cl(Ca)}$ Density Is Not Affected by Heart Failure

Thus far, we found that in human ventricular cells, DAD and $I_h$ are carried predominantly by $I_{NaCa}$, but not by $I_{Cl(Ca)}$. This is at odds with observations made in a number of animal models. In our experiments, ventricular cells were isolated from explanted hearts of patients with end-stage heart failure. We cannot exclude the possibility that heart failure affects $I_{Cl(Ca)}$ density, the more so because heart failure is associated with downregulation of a number of cationic currents31,32 and alterations in Ca$^{2+}$ metabolism.33

In a concluding series of experiments, we determined whether in an animal model, heart failure influences $I_{Cl(Ca)}$ density. We studied $I_{Cl(Ca)}$ in cells isolated from control and failing rabbit hearts using the low-Cl$^-$ pipette solution (E$_{Cl}$ = $-50$ mV). Moreover, the results obtained in rabbit cells were compared with those obtained with failing human cells. To measure $I_{Cl(Ca)}$ we applied voltage-clamp steps (see Methods) in the absence and presence of DIDS. Figure 5A shows typical examples of the current traces recorded at $+50$ mV in a control rabbit (left), a failing rabbit (middle), and a failing human (right) cell. By subtraction of the appropriate traces, the DIDS-sensitive $I_{Cl(Ca)}$ was obtained (Figure 5B).

Figure 5C shows mean $I$-$V$ relationships of $I_{Cl(Ca)}$ of 11 control rabbit, 9 failing rabbit, and 6 failing human cells. In both groups of rabbit cells, the typical bell-shaped $I_{Cl(Ca)}$ $I$-V relationship was found. $I_{Cl(Ca)}$ was not observed in human
ventricular cells. Moreover, the $I_{\text{CCa}}$ density in failing rabbit cells did not differ significantly from that in control rabbit cells. From these data, we conclude that in rabbit, heart failure per se does not necessarily lead to downregulation of $I_{\text{CCa}}$.

Discussion

Overview

The aim of this study was to elucidate the ionic mechanism of DADs and their underlying current, $I_c$, in failing human ventricular cells. We found that the $I_c$ I-V relationship was inwardly directed between $-110$ and $+50$ mV (Figures 2 and 3). Neither changes in $[\text{Cl}^-]$ nor application of DIDS (Figure 3) affected the DADs or $I_{\text{Cl}}$ (Figure 2). $I_c$ was completely abolished, however, by substitution of extracellular Na$^+$ (Figure 4). Because this maneuver blocks $I_{\text{NaCa}}$, we conclude that $I_{\text{NaCa}}$ is the principal ionic mechanism of DADs and $I_c$ in failing human ventricular cells.

Species-Dependency of Ionic Nature of DADs

Our results indicate that in failing human ventricular cells, only $I_{\text{NaCa}}$ contributes to $I_c$. This is similar to findings in ventricular cells of guinea pig\textsuperscript{25,29} but contrasts with observations made in ventricular cells of dog,\textsuperscript{11} rabbit,\textsuperscript{12,13} sheep,\textsuperscript{14} and ferret.\textsuperscript{30} In these species, $I_c$ consisted of both $I_{\text{ClCa}}$ and $I_{\text{NaCa}}$. In our experiments, norepinephrine was present. Adrenergic stimulation enhances $I_{\text{ClCa}}$ amplitude,\textsuperscript{11} thereby potentially favoring $I_{\text{ClCa}}$ detection. Furthermore, we found that the density of $I_{\text{ClCa}}$ in rabbit heart cells is not affected by heart failure (Figure 5). Thus, neither our experimental conditions nor heart failure per se can clarify why $I_{\text{ClCa}}$ cannot be found in our failing human ventricular cell preparation. The simplest explanation is that our failing human heart cell preparation does not possess $I_{\text{ClCa}}$. Indeed, Köster et al\textsuperscript{34} were not able to detect $I_{\text{ClCa}}$ currents in human ventricular cells when they applied caffeine to induce SR Ca\textsuperscript{2+} release, whereas in rabbit Purkinje cells, that same maneuver does activate $I_{\text{ClCa}}$.\textsuperscript{35} Finally, the lack of effect of DIDS application and/or intracellular Cl$^-$ substitution on steady-state membrane currents and action potentials of our failing human ventricular cells (Figures 2 and 3) also implies that in our preparation, both the heart failure–induced, persistently active swelling-induced Cl$^-$ current\textsuperscript{36} and cAMP-dependent Cl$^-$ current ($I_{\text{ClAMP}}$)\textsuperscript{37} are absent. The latter agrees with findings of Oz and Sorota,\textsuperscript{38} who were also not able to detect $I_{\text{ClAMP}}$ in human ventricular cells.

Limitations of Our Study

Animal models contribute much to our understanding of the electrophysiology of failing hearts. They can never completely substitute, however, for experiments with normal human cells. We found that heart failure in rabbits does not significantly alter $I_{\text{CCa}}$. We take that as evidence that heart failure per se cannot account for our inability to detect $I_{\text{CCa}}$ in human ventricular cells of failing hearts. Final proof for the notion that human heart cells do not express $I_{\text{CCa}}$, of course, lies in experiments with normal human heart cells.

Implications of Our Study

The present study demonstrates that in failing human ventricular cells, DADs and their underlying current, $I_c$, are composed virtually exclusively of $I_{\text{NaCa}}$. $I_{\text{NaCa}}$ is activated by spontaneous Ca$^{2+}$ release from SR. This contrasts with observations made in ventricular cells of a number of animal species in which $I_{\text{NaCa}}$ also contributes to the DADs and $I_c$. Our findings question the rationale behind $I_{\text{NaCa}}$ blockade as a therapeutic approach in arrhythmias caused by triggered activity.

Acknowledgments

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References


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