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 Ca\(^{2+}\)-activated Cl\(^{-}\) current (\(I_{\text{Cl(Ca)}}\)) and the Na\(^{+}/Ca\(^{2+}\) exchange current (\(I_{\text{Na(Ca)}}\)) contribute to the transient inward current (\(I_{\text{ti}}\)). \(I_{\text{ti}}\) is responsible for the proarrhythmic delayed afterdepolarizations (DADs). We investigated the ionic mechanism of \(I_{\text{ti}}\) 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. \(I_{\text{tiS}}\) were elicited in the presence of 1 \(\mu\)mol/L norepinephrine by trains of repetitive depolarizations from −80 to +50 mV. DADs were induced in the presence of 1 \(\mu\)mol/L norepinephrine at a stimulus frequency of 1 Hz. \(I_{\text{ti}}\) currents were inwardly directed over the voltage range between −110 and +50 mV. Neither the Cl\(^{-}\) channel blocker 4,4′-diamidino-2,6′-disulphonic acid nor changes in [Cl\(^{-}\)], affected \(I_{\text{ti}}\) or DAD amplitude. This excludes an important role for \(I_{\text{Cl(Ca)}}\). Blockade of Na\(^{+}/Ca\(^{2+}\) exchange by substitution of all extracellular Na\(^{+}\) by Li\(^{+}\), conversely, completely inhibited \(I_{\text{ti}}\). In rabbit, \(I_{\text{Cl(Ca)}}\) 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, \(I_{\text{ti}}\) and DADs in human ventricular cells from failing hearts consist only of \(I_{\text{Na(Ca)}}\). In rabbits, heart failure per se does not alter \(I_{\text{Cl(Ca)}}\) density, suggesting that \(I_{\text{Cl(Ca)}}\) 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

Patients with congestive heart failure have a high incidence of ventricular arrhythmias.\(^1\) They are at increased risk of sudden death resulting from ventricular tachycardia and ventricular fibrillation.\(^2\) It was recently demonstrated that these potentially lethal arrhythmias in patients with end-stage idiopathic dilated cardiomyopathy arise in the subendocardium or subepicardium by a focal nonreentrant mechanism.\(^3\) Triggered activity developing from either early afterdepolarizations (EADs) or delayed afterdepolarizations (DADs) may play a pivotal role in the initiation of these rhythm disturbances.\(^3\)

The afterdepolarizations are “oscillations” in membrane potential. EADs occur during the action potential, whereas DADs occur after completion of the action potential.\(^4\) In ventricular cells from end-stage failing human hearts, phase-2 EADs are due to reactivation of the L-type Ca\(^{2+}\) current.\(^5\) So far, the mechanism of DADs has been investigated in animal models only. These studies showed that DADs are provoked by high heart rates under conditions in which [Ca\(^{2+}\)] is elevated.\(^6\) The current causing DADs is called the transient inward current (\(I_{\text{ti}}\)).\(^7\) \(I_{\text{ti}}\) is activated by spontaneous Ca\(^{2+}\) release from the sarcoplasmic reticulum (SR).\(^6\) \(I_{\text{ti}}\) proves to be a heterogeneous current. One component flows through nonselective cation channels.\(^8\) Current mediated by Na\(^{+}/Ca\(^{2+}\) exchange (\(I_{\text{Na(Ca)}}\)) constitutes a second component.\(^8\) More recently, a third component was identified. Evidence has accumulated that the Ca\(^{2+}\)-activated C\(^{-}\) current (\(I_{\text{Cl(Ca)}}\)) also contributes to both \(I_{\text{ti}}\)\(^9\)-\(^12\) and DADs.\(^13\)-\(^15\) We recently demonstrated in sheep heart cells that blockade of \(I_{\text{Cl(Ca)}}\) reduced the DAD amplitude sufficiently to prevent 50% of the DADs from reaching the threshold for triggering action potentials.\(^14\) This observation suggests that \(I_{\text{Cl(Ca)}}\) blockade may be a promising therapeutic strategy in those instances in which DADs contribute to the genesis of arrhythmias; that is, of course, only when \(I_{\text{Cl(Ca)}}\) also plays a role in \(I_{\text{ti}}\) and DADs in human ventricular cells. In this article, we address this issue.

Here, we report that \(I_{\text{ti}}\) and DAD in ventricular cells isolated from human end-stage failing hearts are caused by \(I_{\text{Na(Ca)}}\) only. Furthermore, we show that in a rabbit model, heart failure does not lead to disappearance of \(I_{\text{Na(Ca)}}\). Our results suggest that failing, and presumably also nonfailing, human ventricular cells do not possess \(I_{\text{Cl(Ca)}}\) channels. Our findings
question the rationale behind $I_{\text{Cl(Ca)}}$ blockade as a therapeutic approach in arrhythmias caused by triggered activity based on DADs.

### Methods

#### 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.16

#### Rabbit Ventricular Cells

Heart failure was induced in New Zealand White rabbits by volume and pressure overload.13 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.17 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. Ventricular cells were isolated from the left ventricle by enzymatic dissociation.16 Cells isolated from failing hearts were served as control group. Animal care was in accordance with institutional guidelines. Ventricular cells were isolated from the left ventricle by enzymatic dissociation.16

#### 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 5.0 (pH adjusted to 7.4 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.

**Figure 1.** A, Voltage-clamp protocol for eliciting $I_{\text{tp}}$. B, Current recording made during this protocol in a human ventricular cell. Arrow, $I_{\text{tp}}$. Inset, Protocol for measuring $I_{\text{tp}}$ amplitude. C, Effects of 0.2 mmol/L tetracaine on occurrence of $I_{\text{tp}}$ (left) and DAD (right).
Results

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

We first asked the question whether in human ventricular cells \( I_{\text{ti}} \) and DADs are activated by spontaneous SR Ca\(^{2+}\) release. We tested the effects of 0.2 mmol/L tetracaine and 5 mmol/L caffeine, drugs known to inhibit spontaneous SR 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 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 \( I_{\text{ti}} \) and its sensitivity to intracellular Cl\(^{-}\) substitution. We reasoned that if \( I_{\text{Cl(Ca)}} \) contributes to \( I_{\text{ti}} \), then a change in [Cl\(^{-}\)]\(_{\text{i}}\) has to alter the \( I_{\text{ti}} \) I-V relationship. We applied the aforementioned train protocol, but now directly followed by 17 voltage-clamp steps of 3-second duration and ranging from -110 to +50 mV with 10-mV increments (Figure 2A, inset). Figure 2A shows typical current traces recorded at the membrane potentials indicated. In this and 4 other experiments, the low-Cl\(^{-}\) pipette solution was used. The corresponding Cl\(^{-}\) reversal potential (E\(_{\text{Cl}}\)) was calculated at -50 mV, which is the physiological E\(_{\text{Cl}}\) value in cardiac cells.\(^{22}\) For 4 other cells, the high-Cl\(^{-}\) pipette solution was used, for which E\(_{\text{Cl}}\) was 0 mV. Figure 2B shows the I-V relationships of \( I_{\text{ti}} \) measured with low and high [Cl\(^{-}\)]\(_{\text{i}}\). Irrespective of [Cl\(^{-}\)]\(_{\text{i}}\), \( I_{\text{ti}} \) was inwardly directed at all potentials 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 [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 \( I_{\text{ti}} \) 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-Cl\(^{-}\) pipette solution (E\(_{\text{Cl}}\)= -50 mV). Figure 3A shows typical examples of \( I_{\text{ti}} \) measured at -80 and +50 mV in the absence and presence of DIDS. The drug had no effect.

Statistics

Action potential characteristics were derived from 10 consecutive action potentials and averaged. Microsoft Excel software was used for statistical analysis of the data. Values are expressed as mean±SEM and considered significantly different at a value of P<0.05 in ANOVA or Student’s t test.
Figure 5. A, Superimposed current traces elicited by voltage-clamp steps from −50 to +50 mV in absence and presence of DIDS in a control rabbit cell (91 pF), failing rabbit cell (290 pF), and failing human cell (265 pF). B, DIDS-sensitive currents. C, I-V relationship of \( I_{\text{Na/Ca}} \) in ventricular cells from control rabbit (\( n=11 \)), failing rabbit (\( n=9 \)), and failing human (\( n=6 \)) hearts.

Figure 3C shows mean I-V relationships of 4 cells before and after substitution of all extracellular Na\(^+\). After contractions were still visible, however, \( I_n \) in failing human ventricular cells ruptured. Figure 4B shows the mean \( I_n \) I-V relationship of 4 cells before and after the maneuver. After substitution of Li\(^+\) for Na\(^+\), \( I_n \) was abolished at all potentials. Inhibition of Na\(^+\)/Ca\(^{2+}\) exchange and the consequent further loading of the sarcoplasm with Ca\(^{2+}\) prevents stable current-clamp recordings. For this reason, we could not evaluate the effect of Li\(^+\) on DAD formation. Nevertheless, from these data, we conclude that \( I_{\text{Na/Ca}} \) plays a dominant role in \( I_n \) and DAD formation in human ventricular cells of failing hearts.

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

Thus far, we found that in human ventricular cells, DAD and \( I_n \) are carried predominantly by \( I_{\text{Na/Ca}} \), but not by \( I_{\text{Cl(Ca)}} \). 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 \( I_{\text{Cl(Ca)}} \) density, the more so because heart failure is associated with downregulation of a number of cationic currents\(^{31,32}\) and alterations in Ca\(^{2+}\) metabolism.\(^{33}\)

In a concluding series of experiments, we determined whether in an animal model, heart failure influences \( I_{\text{Cl(Ca)}} \) density. We studied \( I_{\text{Cl(Ca)}} \) in cells isolated from control and failing rabbit hearts using the low-Cl\(^-\) pipette solution (E\(_{\text{Cl}}\) = −50 mV). Moreover, the results obtained in rabbit cells were compared with those obtained with failing human cells. To measure \( I_{\text{Cl(Ca)}} \) 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 \( I_{\text{Cl(Ca)}} \) was obtained (Figure 5B). Figure 5C shows mean I-V relationships of \( I_{\text{Cl(Ca)}} \) of 11 control rabbit, 9 failing rabbit, and 6 failing human cells. In both groups of rabbit cells, the typical bell-shaped \( I_{\text{Cl(Ca)}} \) I-V relationship was found. \( I_{\text{Cl(Ca)}} \) was not observed in human


dominate role in the \( I_n \) I-V relationship of 4 cells. Figure 3C and 3D shows that the \( I_{\text{Cl(Ca)}} \) relationship of 4 cells before and after substitution of all extracellular Na\(^+\) by Li\(^+\) (4 cells).

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

**Dominant Role for \( I_{\text{Na/Ca}} \) in \( I_n \) and DAD Formation in Human Ventricular Cells**

A salient feature of the \( I_n \) 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 \( I_n \). Na\(^+\)/Ca\(^{2+}\) exchange can support inwardly and outwardly directed currents.\(^{24}\) During spontaneous SR Ca\(^{2+}\) release under voltage-clamp conditions, however, \( I_{\text{Na/Ca}} \) 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 \( I_n \) 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, \( I_n \) decreases (Figures 2B and 3B). This behavior is most likely related to voltage-dependency of \( I_{\text{Na/Ca}} \) 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,\(^{29}\) which will result in a smaller \( I_n \).

Next, we tested the hypothesis that \( I_{\text{Na/Ca}} \) contributes to \( I_n \). We inhibited Na\(^+\)/Ca\(^{2+}\) exchange by replacing extracellular Na\(^+\) with equimolar amounts of Li\(^+\). Li\(^+\) permeates through Na\(^+\) channels and nonselective cation channels\(^{28}\) 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\(_{\text{Cl}}\) = −50 mV) was used. Figure 4A shows a typical example of the effects of Li\(^+\) on \( I_n \). Both at −80 and at +50 mV, an inwardly directed \( I_n \) 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...
ventricular cells. Moreover, the $I_{\text{CaL}}$ 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 $I_{\text{CaL}}$.

Discussion

Overview

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

Species-Dependency of Ionic Nature of DADs

Our results indicate that in failing human ventricular cells, only $I_{\text{NaC}}$ contributes to $I_{\text{CaL}}$. This is similar to findings in ventricular cells of guinea pig but contrasts with observations made in ventricular cells of dog, rabbit, sheep, and ferret. In these species, $I_{\text{NaC}}$ consisted of both $I_{\text{CaL}}$ and $I_{\text{NaC}}$. In our experiments, norepinephrine was present. Adrenoceptor stimulation enhances $I_{\text{CaL}}$ amplitude, thereby potentially favoring $I_{\text{CaL}}$ detection. Furthermore, we found that the density of $I_{\text{CaL}}$ 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 $I_{\text{CaL}}$ cannot be found in our failing human ventricular cell preparation. The simplest explanation is that our failing human heart cell preparation does not possess $I_{\text{CaL}}$. Indeed, Köster et al were not able to detect $I_{\text{CaL}}$ currents in human ventricular cells when they applied caffeine to induce SR Ca$^{2+}$ release, whereas in rabbit Purkinje cells, that same maneuver does activate $I_{\text{CaL}}$. 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 and cAMP-dependent Cl$^-$ current ($I_{\text{NaClAMP}}$) are absent. The latter agrees with findings of Oz and Sorota, who were also not able to detect $I_{\text{NaClAMP}}$ 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 $I_{\text{CaL}}$. We take that as evidence that heart failure per se cannot account for our inability to detect $I_{\text{CaL}}$ in human ventricular cells of failing hearts. Final proof for the notion that human heart cells do not express $I_{\text{CaL}}$ 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, $I_{\text{CaL}}$, are composed virtually exclusively of $I_{\text{NaC}}$. $I_{\text{NaC}}$ 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 $I_{\text{NaC}}$ also contributes to the DADs and $I_{\text{CaL}}$. Our findings question the rationale behind $I_{\text{NaC}}$ blockade as a therapeutic approach in arrhythmias caused by triggered activity.

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